

THE EXTRA PHARMACOPŒIA

MARTINDALE

Twenty-second Edition
IN TWO VOLUMES



VOLUME II

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PREFACE

Each of the first fourteen editions of the *Extra Pharmacopœia* was published in one volume and it was in 1912 that the first edition in two volumes was published. It was realised at that time by the author that the ever-increasing amount of information on compounds and preparations used by the physician and the pharmacist was too great to be issued conveniently in one volume. The second volume of the Fifteenth Edition contained 370 pages and the matter collected and considered suitable for recording has increased steadily during the last thirty years; this second volume of the Twenty-second Edition contains 1217 pages. Since the publication of the second volume of the Twenty-first Edition in October 1938, despite the conditions prevailing during a world war, much new information has been gathered from the continued progress of research in medicine and chemistry and, although the general scope of the volume has undergone little change, substantial modifications of the matter in the previous edition have been made and a considerable proportion of new matter has been added. This second volume deals with the numerous subjects ancillary to those dealt with in the first volume and will continue to provide the reader of the *Extra Pharmacopœia* with up-to-date information on such matters as the methods by which the purity of medicinal substances is maintained, the analysis and composition of some of the principal foods, the application of chemistry as an aid to clinical diagnosis and the bacteriology of certain diseases. It includes also matter on numerous other subjects with which physicians, chemists and pharmacists should be acquainted in the ever-widening sphere of studies for the improvement of health, the relief of pain and the cure of disease.

Analytical Addenda

The requirements of the British Pharmacopœia, 1932, and of the British Pharmaceutical Codex, 1934, are summarised in this section and special attention has been given to comments and abstracts from published papers which have a bearing on these official standards. All the modifications and changes in the addenda to the *B.P.* issued since 1938 have been incorporated, as well as the new matter and amendments in the six supplements to the *B.P.C.* The assays and standards of the *United States Pharmacopœia* and the *American National Formulary* have been revised in accordance with the *U.S.P. XII* and the *N.F. VII* which became official on November 1st, 1942, and the standards required by the most recent issues available of other national pharmacopœia are also included. Comparisons of the standards for the same substances in these different authorities show clearly the divergencies of opinion which exist concerning individual

methods and emphasize the need for closer co-operation between the responsible parties of the various countries. Valuable information to analysts is provided by the inclusion of assay processes for many medicinal substances in the form of tablets from the *U.S.P.* and the *N.F.* In the 21st Edition reference was made to the Second Report of the International Commission dealing with the analysis of proprietaries and to the attention it gave to the standardisation of substances in tablets, but so far no addendum to the *B.P.* or supplement to the *B.P.C.* has been published containing monographs comparable with those of the United States authorities. Similarly, references are made to valuable work done in America by the Association of Official Agricultural Chemists, to the specifications for numerous materials produced by the British Standards Institution and to the agreed methods produced by Sub-Committees set up by the Society of Public Analysts. As in previous editions, references to or abstracts of papers of direct value which have appeared in the various pharmaceutical, medical and chemical publications have been included.

The following are a few of the important subjects dealt with in this section:—

ACIDUM ACETYSALICYLICUM. In the 21st Edition reference was made to the method of the A.O.A.C. for the determination of aspirin in tablets and to processes recommended for determining free salicylic and acetic acids, but there were no officially standardised tablets. Suggestions have been made to include standards for tablets of all the commoner medicinal substances in either the *B.P.* or the *B.P.C.*, but none have been issued up to the present. The *U.S.P. XII* and the *N.F. VII* contain many of these tablets and here we would draw attention to the *U.S.P.* monograph on aspirin tablets as an example of methods summarised in this edition which will be useful as a guide to analysts in this country until such time as the authorities here are ready to publish British standards. The *U.S.P.* does not prescribe a definite size or shape of tablet but requires the bulked tablets to contain from 95 to 105% of the labelled amount of pure $C_9H_8O_4$. In general the standards are set on the substances assayed and therefore there are no further allowances to be made for the purity of ingredients or loss in manufacture. Undue variations in results due to different methods of sampling or to varying numbers of tablets in the samples are avoided by taking 20 tablets for the assays. Evidently tablets are not necessarily manufactured from pure material, but they must contain on the average, within defined limits, a quantity of chemically pure material equivalent to the stated amount of drug present. Considerable variation in size is permitted and the rate of disintegration, shape, colour, the presence of impurities and proprietary marks have not been considered of sufficient importance to warrant control.

ACIDUM BORICUM. The standards for surgical dressings are included and it will be noted that owing to the need for economy

in the use of boric acid the strengths of official preparations have been reduced—Boric Lint *B.P.C.* from 40 to 5% and Boric Ointment *B.P.* from 10 to 1%. The simple methods used for determining boric acid in a paraffin base may require slight modification when dealing with a wool wax base and a much smaller percentage of boric acid.

ACIDUM HYDROCYANICUM. Reference is made to the specifications for cyanide insecticides, and here is added the first of a number of abstracts included in this section from leaflets issued by the Department of Scientific and Industrial Research on Methods for the Detection of Toxic Gases in Industry which can be obtained from His Majesty's Stationery Office. Hydrocyanic acid is widely used as a fumigant and its vapour may also be met with in dangerous concentrations in certain industries.

ACIDUM MANDELICUM. The standards of the *B.P.* and *U.S.P.* are compared and the *U.S.P.* standard is included for the calcium salt which is sometimes preferred owing to its freedom from unpleasant taste when mandelic acid is administered in the treatment of infections of the urinary tract.

ACONITUM. This drug has been dropped from the *U.S.P.* and transferred to the *N.F. VII* so that the controversy concerning standardisation is not now so acute. The American drug is still standardised biologically, but in Great Britain a new chemical assay has been devised by a sub-committee appointed by the Society of Public Analysts and this method which is outlined will be sufficient to meet the needs of analysts who have to standardise galenicals or to determine the alkaloids of aconite under the Pharmacy and Poisons Act, 1933, or the Pharmacy and Medicines Act, 1941.

ALCOHOL TRIBROMOETHYLICUM. Bromethol is the solution of tribromoethyl alcohol in amylene hydrate used as a basal anæsthetic for rectal administration and the standards of the *B.P.* and *U.S.P.* for both substances are given. It is difficult to understand why these two standards should differ so appreciably; some of the tests for purity for both tribromoethanol and amylene hydrate may produce the desired results and both assays for tribromoethanol may be considered dependable, but why in two English-speaking countries should they be so differently expressed? The *B.P.* requires 99% of tribromoethanol and the *U.S.P.* only 98% and the specific gravities and boiling ranges are not comparable. Incidentally, analysts who are accustomed to checking official boiling ranges have to use a more practicable procedure than that officially prescribed.

ALCOHOLIA LANÆ. This is the *B.P.* Latin name for the mixture of wool alcohols prepared by the saponification of wool fat which is a non-proprietary emulsifying agent now used in place of paraffin bases for making many official ointments. In some cases absorption of the active ingredient from the hydrous base is more rapid and in war-time a great economy in paraffins

is effected. The standard required is included and the official digitonin assay is outlined.

ALUMINIUM. In this section the two new *B.P.* kaolins and the standards set for limits of particle size which are determined by finding the specific gravities of suspensions prepared under controlled conditions are referred to. Light kaolin is to be used for internal administration and the heavy variety for making kaolin poultice. For further particulars regarding the control of these two substances the recent paper by Page (*Quart. J. Pharm.*, 1943, 1) should be consulted. Bentonite, the American native aluminium silicate, is also referred to.

ARGENTI PROTEINAS. The nomenclature of the two silver proteates of the *U.S.P.* and that of the corresponding substances in the *B.P.* and *B.P.C.* have not reduced the confusion between therapeutic activity and silver content; adjectives indicating activity are out of place when associated with chemical names. This confusion has evidently spread to the compilers of the *U.S.P. XII* and the amusing error made by including the same test for distinction between the two substances in both monographs needs early correction.

BARBITONUM. The standards for several new barbiturates of the *B.P.*, *B.P.C.*, and *U.S.P.* are described, including those for Hexabarbitone, Soluble Hexabarbitone, Phemitone and Soluble Phenytoin. Attention is given to the several standardised tablets of the *U.S.P.* The lack of uniform nomenclature for all these important hypnotics is a regrettable feature and provides another example of the need for that co-operation which was stressed in the second report of the International Commission. "Phenobarbitonum Solubile" *B.P.* is required to contain not less than 95% of $C_{12}H_{11}O_3N_2Na$, whereas "Phenobarbitalum Sodicum" *U.S.P.* may contain 7% of moisture and the dried substance from 89 to 91.5% of $C_{12}H_{11}N_2O_3$.

BELLADONNA. Reference is made to the admission to the *B.P.* of Indian belladonna leaf and root derived from *Atropa acuminata* (Royle), syn. *Atropa lutescens* (Jacquem) which may now be used for the manufacture of galenicals. The assay is altered so as to eliminate volatile bases obtained, but there does not appear to be any agreement as to the best way of attaining this, and the standards do not seem to be very suitable for shipments exported to Great Britain.

CAFFEINA. In this section standards and assay processes for caffeine, theobromine and theophylline and several compounds of these three ureides are outlined, including theophylline ethylenediamine and the tablets of the *U.S.P.* and *N.F.* In America the process of Self and Rankin appears to give erratic results and the *U.S.P.* has adopted the silver method of Stevens and Wilson, which undoubtedly gives low results, in preference to Warren's useful modification of Reimer's process.

CARBO. The standards of the *B.P.C.* and *U.S.P.* for medicinal charcoal are compared, but there is still no pharmacopœial

specification for this important material. Attached to this section are useful short references to the leaflets issued by the Department of Scientific and Industrial Research on the detection of carbon monoxide and phosgene which may occur in dangerous proportions in certain industries.

CINCHONA. In the section on Cinchona is included a tabulated summary of the compositions and standards for all the official quinine salts and the standards for other cinchona alkaloids of the *B.P.*, *B.P.C.* and several national pharmacopœias. Recent orders under the Defence Regulations control the sale and use of cinchona, cinchona products and synthetic substitutes. The principal synthetic substitutes are mepacrine hydrochloride and methanesulphonate and pamaquin, which are used for their action upon malarial parasites; their official standards are summarised in comparison with the corresponding American substances, quinacrine hydrochloride and pamaquine naphthoate.

DERRIS. The variation in activity and rotenone content of different supplies of this important insecticide continues to receive attention from experts and variable results are frequently obtained in different laboratories. The work of Coombes, Martin and Harper is referred to and a process recommended for inclusion in the *B.P.C.* and based upon a paper published by them is described.

DIGITALIS. The biological methods for the standardisation of powdered digitalis and tincture of digitalis are summarised. The reference standard of the *U.S.P.* is now equivalent in potency to the standard adopted by the League of Nations Commission, and the *U.S.P. XII* contains a standardised tablet of the leaf. Digoxin, the important glycoside obtained from the leaves of *Digitalis lanata* and which is of particular value in the treatment of auricular fibrillation, does not require biological standardisation and chemical tests for purity are now described in the *B.P.*; until August, 1945, digoxin is controlled by patents in Great Britain and Northern Ireland.

EPHEDRA. The *B.P.* standard for ephedrine monohydrate and the *B.P.C.* standard for anhydrous ephedrine are compared with that of the *U.S.P. XII* which includes both forms. Tablets of the sulphate and hydrochloride are standardised in the *U.S.P.* and *N.F.* respectively; summaries are also given of the new *B.P.C.* standards for amphetamine and amphetamine sulphate which in their local effects resemble adrenaline and ephedrine.

ERGOT. Years have passed since the classical experiments by Chassor Moir which resulted in ergometrine being accepted as the most important constituent of ergot. To-day in the 6th addendum to the *B.P.* this drug and its official preparations are standardised not only on their total alkaloid but also on their ergometrine content. The old standard of 0.05% of total alkaloid for ergot is replaced by one requiring 0.2% of total alkaloid of which not less than 15% is water-soluble alkaloid calculated as ergometrine and the drug is assayed by the well-tried

method of Hampshire and Page. Some chemists are of the opinion that the determination of water-soluble alkaloids is not a measure of the ergometrine content and that the percentage of true ergometrine may be below 10% of the total alkaloid. A good ergot usually contains over 0.25% of alkaloids and often 20% of this is water-soluble. The *U.S.P. XII* still uses the pedigree Leghorn cock method with ergotoxine ethanesulphonate as control.

FERRUM. This section contains many useful references to iron determinations in medicinal preparations with abstracts of improved procedures for several compounds and for the determination of small amounts of impurities such as lead and copper.

GOSSYPIUM. The 1st Supplement to the *B.P.C.* contains new surgical wound dressings and a summary of its requirements is given under this heading, together with references to alternative specifications for battiste and jaconet and the new waterproofed rayon which is used in the application of hypochlorite solution for the treatment of burns.

IPECACUANHA. The official standards have again been reduced to admit the poorer type of Minas root to be used for powdered ipecacuanha and other galenicals. The effect of this remains to be seen; with the high prices prevailing there may be a demand for still poorer material and a further request for a lower standard.

MAGNESII TRISILICAS. This substance has found a place in the treatment of peptic ulcers, but in some quarters doubt is expressed concerning its efficacy. Some details are given of the *B.P.* and *B.P.C.* requirements and reference is made to the *U.S.P.* tablets which are standardised for limit of free alkali, acid-consuming capacity and adsorptive power.

ESTRADIOL. Following the introduction of specific sex hormones this section has been completely rewritten. Stilbæstrol of the *B.P.* and the substances of the *U.S.P.* are compared, together with the several hormones of the 3rd Supplement to the *B.P.C.*, including œstradiol, œstradiol benzoate, œstrone, œstrone benzoate, progesterone, testosterone and testosterone propionate. Possibly by the time the next edition is published some more useful therapeutic applications may have been discovered for some of these mysterious medicaments.

SACCHARIN. In a time of sugar shortage this substance is an important commodity and the standards of the *B.P.C.* and *B.P.* for saccharin and soluble saccharin respectively are compared with those of the *U.S.P.* for saccharin and "saccharinum sodicum". Abstracts of newer methods for the determination of saccharin and dulcin in foodstuffs and tablets are also given.

SULPHANILAMIDUM. Following the discovery of the value of the sulphonamide group of chemicals in the treatment of streptococcal and pneumococcal infections several new specifications have been included in the national pharmacopœias, among them being sulphanilamide, sulphapyridine and sulphathiazole, which

are also included as tablets in the *U.S.P. XII*. The assays are compared and several abstracts of papers on recent investigations have been added.

VITAMINS. Ascorbic, nicotinic and pantothenic acids, aneurine hydrochloride, riboflavin and other substances related to the naturally occurring food factors are described in the later sections dealing with nutrition and vitamins.

Proprietary Medicines

The section gives a brief historical review of the various efforts, made over a period of years, to secure more adequate legislative control over the advertising and distribution of proprietary medicines. Since the previous edition was published the passing of the Pharmacy and Medicines Act 1941 has modified many of the previously existing practices. The Act, so far as it concerns proprietary medicines, secures two main objects; it repeals the Medicine Stamp Duties and it imposes certain restrictions on the sale of medicines. Medicines are no longer subject to stamp duty but all articles sold with a recommendation as to their therapeutic value in the treatment of human ailments must bear a quantitative disclosure of composition. The Act endeavours to maintain, as far as possible, the position which existed when "destamping" had become the almost invariable rule. In addition, the advertising of remedies for the treatment of certain diseases is prohibited. Section 12 creates a group of "authorised persons" permitted to sell recommended medicines and limits the class of medicines which may be sold by drug sellers not within one of the named classes. Definite rules are laid down for the method to be adopted in naming the ingredients of a preparation and although the compositions of a large number of preparations have been disclosed it must not be assumed that the correct form of disclosure has been adopted by the maker in every case. Revision of labels to bring them into line with the legal requirements is still in progress and it is possible that revised forms of disclosure will be adopted from time to time as misleading names are discovered and when reprinting is undertaken by the manufacturers.

The list of proprietary medicines included has been completely revised and now contains approximately 750 of the most widely advertised remedies. The so-called ethical proprietaries have been transferred to Volume I and the list now contains only those preparations which are popularly described as "patent" medicines. The disclosure of composition has abolished the principal criterion by which ethical preparations and proprietary medicines were distinguished and for all practical purposes secret remedies are to-day non-existent. Ethical preparations which are usually included in Volume I cannot therefore be clearly distinguished from the non-ethical by reference to the amount of information available as to their composition. The division is now based upon the method of distribution, i.e., upon whether a preparation is sold to the public by advertisement and without reference to the

requirements of the individual patient or whether it is a product recommended or prescribed for use under medical supervision. In each case the name of the manufacturer, the principal claims made for the preparation and the formula or composition is taken from the label of the container or from the accompanying literature. The list may be useful to doctors who seek information concerning preparations which their patients have been in the habit of taking. It reveals an extremely interesting situation and emphasizes the need for immediate action by the responsible authorities. Although war conditions may in certain cases provide a plausible excuse, it seems unreasonable a year after the Pharmacy and Medicines Act came into force that it should be possible to purchase proprietaries for which the composition is not disclosed or is disclosed in a manner not in accordance with legal requirements. That so many useless "medicines" containing ingredients which in war-time must be imported or are required more urgently for other purposes is to many persons beyond comprehension. Furthermore, it will be interesting to see for how long unjustifiable claims are permitted to be made for preparations containing only ingredients which have no useful therapeutic effect. In many cases the effect can only be psychological and due to the extraordinary claims or to the illegal or pseudo-scientific terminology by which excipients, vehicles, flavours and colouring matters are presented as modern therapeutically active and important constituents.

Nomenclature of Organic Compounds

The basic framework of this section cannot alter, but its utility has been extended to include most of the medicinal compounds which occur in the *B.P.*, the *B.P.C.* and their addenda and supplements. From July 1st, 1942, the formula or composition for substances recommended as medicines must be disclosed on their containers or labels. If the constituents are in the *B.P.* or *B.P.C.*, the official name, abbreviation or synonym must be used. If such be not the case "the accepted scientific name or other name descriptive of the true nature of the substance, constituent or ingredient" may be used. For chemical compounds the term "accepted scientific name" referred to in the Pharmacy and Medicines Act, 1941, may be interpreted as a non-ambiguous, systematic name which would disclose to a competent chemist the chemical structure or identity of the ingredient. It is obvious that this new requirement focuses attention on the necessity for a systematic nomenclature.

Chemotherapy

This section is not limited to a consideration of only those chemicals which combat invading organisms, since the true interpretation of the word "chemotherapy" includes the action of all chemicals used in therapy and excludes no substance the therapeutic activity of which is related to its chemical structure.

The matter has been revised to include the work of the last few years and reference is made to the new theories of indirect action whereby the substance is thought to exert its effect by interfering with an essential enzyme process or an essential metabolite. The development of the sulphonamide drugs is brought up-to-date by the inclusion of the main compounds of this type in use to-day and some indication is given of the objects and aims for future research in the series. The remarkable work of the last few years resulting in a knowledge of the structure of the sterols, bile acids, cardiac glycosides, calciferol and the steroid hormones provides a striking instance of greatly differing effects produced by variation on a single theme (the cyclopentenophenanthrene skeleton). In this connection the work which led to the introduction of stilboestrol and hexoestrol into medicine and to the discovery of the first synthetic substances possessing activity similar to that of desoxycorticosterone is explained from a chemotherapeutic point of view.

Recognition of Organic Substances

The systematic scheme for the recognition of organic medicinal compounds and the table of corroborative tests remain in the same form, but have been improved by the addition of many new substances including those recently added to the *B.P.* and *B.P.C.*, among which will be found some of the newer barbiturates, anæsthetics, sulphonamides, vitamins and hormones.

Hydrogen Ion Concentration

The theoretical considerations underlying the determination of hydrogen ion concentration and the advantages and disadvantages of the various methods of determination have been retained with an extended list of applications of *pH* measurement and control. The tabulation of the *pH* of solutions of common substances has been extended to include the more important of the newer therapeutic agents.

Oxidation-Reduction Potentials and Polarographic Analysis

The section on oxidation-reduction potentials remains substantially unaltered but the recent increasing use and application of electro-oxidation and electro-reduction to analytical problems by means of current-voltage studies has led to the inclusion of a new section on Polarographic Analysis. Invented in 1925 by Heyrovsky, the polarograph is an instrument by means of which current-voltage curves may be obtained automatically and recorded photographically, the recorded curves being termed polarograms. The theoretical considerations underlying polarography are discussed, the technique of the method is described and the many applications are indicated. Inorganic analyses have been more widely studied but the method has also been applied to many problems of analyses involving organic compounds. The method is specially suitable for small concentrations and is applicable to

values of a large number of foods have been added. As in previous editions sections dealing with particular foodstuffs such as milk and milk products, flour and bread, etc., though dealt with under separate headings, are regarded as forming part of the wider subject of nutrition, while a new section on Food Substitutes has been added for the purposes of information.

MILK AND MILK PRODUCTS. The extensive section on milk has been rearranged and clarified as far as possible and a few minor additions inserted. A new monograph on cheese has been introduced and the section is followed by revised accounts of the composition and analysis of margarine and other foods, including jam and vinegar. Reference is made to the work of Edwards and Nanji on the differentiation of the various types of vinegar by analytical methods. The information on food preservatives and colouring agents is retained, but it has not been considered necessary to retain the short monographs on mould inhibition by preservation and on the so-called putrefactive bases.

FLOUR AND BREAD. The monograph on flour and bread has been re-written, particularly from a nutritional standpoint. War-time restrictions on the types of flour which may be milled have revived the controversy concerning the respective nutritional values of white and brown bread and the increased knowledge now available may have important results in post-war practice. Other starch foods have been included in this section and the effects of different forms of cooking on the nutritional value of potatoes have been summarised.

FOOD SUBSTITUTES. A useful general account of the composition and control of war-time food substitutes has been included. During the past three years public analysts have investigated the composition of new articles purporting to be food substitutes, of which some thousands have been offered to the public. Few of these possessed any intrinsic value and since the Ministry of Food took over the control of the manufacture and retail sale of these preparations only about three hundred have survived.

FOOD LEGISLATION. The principal provisions of the Food & Drugs Act, 1938, are concisely arranged for reference, together with a list of Regulations made under the Act. During the war almost all articles of food are subject to provisions of Statutory Rules & Orders issued by the Ministry of Food; the most important of these are summarised under the title "Alterations in the Composition of Food due to War." Since going to press with this section of the text a further Order has been issued (S.R. & O., 1943, No. 1553) regulating the labelling and advertising of foods, with particular reference to the quantitative disclosure of the mineral and vitamin contents of foods and the restriction of claims or suggestions in advertisements regarding the presence of these substances.

Vitamins

Research work on the vitamins shows no signs of slackening; indeed, it would appear to have been stimulated by war-time

needs. It is much to be deplored that the valuable and painstaking work of scientific and clinical investigators should have given rise to one of the worst examples of commercial exploitation ever perpetrated on the public. The layman has been made aware, through various channels of information, that authentic claims may justifiably be made for the prophylactic and curative properties of a number of the vitamins. What he does not always appreciate is that these clinical applications refer only to the use of individual vitamins in certain specific conditions and that an adequate scheme of dosage under medical supervision is essential to their successful employment. He is thus easily persuaded to waste his money on one or other of the innumerable vitamin preparations with which the market has been flooded in recent years the majority of which, in spite of the scientific appearance of their formulæ and the apparent plausibility of their claims, have little or no therapeutic value. Since the last edition of this volume the chemical composition of a number of the vitamins has been determined and some of them (or their therapeutic equivalents) have been synthesised including vitamins B₁, B₂, C, E and K. For the convenience of readers the vitamin classification is still adhered to, the chemical names of the substances—e.g., aneurine hydrochloride, riboflavin, ascorbic acid, tocopheryl acetate and menaphthone—appearing as subsidiary headings. Many of these substances have been given official recognition in one or other of the *B.P. Addenda* and in the *U.S.P. XII* and information from these publications has been incorporated in the appropriate monographs. The vitamin B complex has been the subject of intensive research work during the past few years and in addition to aneurine hydrochloride and riboflavin there are now available commercially: nicotinic acid and its amide (both *B.P.* substances), pyridoxine (vitamin B₆) and pantothenic acid (usually supplied as calcium pantothenate). Abstracts from the literature dealing with the chemistry, the determination and, in the case of the two last-mentioned, the clinical work on these substances are included. Menaphthone (known in America as menadione) is the synthetic therapeutic equivalent of vitamin K; methods both for the biological and chemical determinations are given. Both menaphthone and acetomenaphthone (the salt used for oral administration) are now *B.P.* The existence of a "permeability vitamin," vitamin P, would appear to have been finally confirmed quite recently by an American worker (R. H. Rigby) who has produced from orange hesperidin a permanently water-soluble substance known as methyl chalcone which affords protection against capillary fragility. The section on vitamins concludes with a new table, especially compiled for this work, giving the average vitamin contents (A, B₁, B₂, C and D) of a very wide range of foodstuffs and the use of this table in conjunction with the table giving the composition and caloric values of foods should prove of assistance in the drawing up of a balanced diet to meet the requirements either of health or disease.

Sterilisation

As a result of recent research work, processes for the sterilisation of solutions for parenteral injection have been revised in the 4th Addendum to the *B.P.* A new process of sterilising at a temperature of 98° to 100°C with the aid of a bactericide has been introduced which greatly facilitates the preparation of sterile solutions of many substances which are too unstable to withstand autoclaving. The process of tyndallisation has been deleted as untrustworthy for the usual type of solution for parenteral injection though it is quite suitable for nutrient media. Much attention has been given during recent years to methods for the sterilisation of surgical dressings, following a report of a heavy tetanus infection in cellulose wadding dressings and of the occurrence of tetanus and welchii spores in sanitary pads and even in so-called "sterile" accouchement sets. Some account of this work has been included.

Disinfectants

This section has been completely re-written and considerably extended to include many new types of disinfectants. Experience of war wounds has increased our knowledge of the use of antiseptics in wounds very considerably and an extended account of this work is given. The important developments in the use of colloidal-mists or aerosols for the sterilisation of the atmosphere, as in air-raid shelters, operating theatres and isolation wards, are included.

Radiology and Electrotherapy

The article on the use of galvanic, faradic and sinusoidal currents for the treatment of disease, which was rewritten for the twentieth edition, was largely based on the results of the treatment carried out in the electrical department of one of the big London hospitals. The article has since been revised and brought up to date and in the present edition there is added an account of the method of treating schizophrenia and some other types of mental illness by means of electrically induced convulsions as originally described by Cerletti and Bini. This method is now employed in several mental hospitals and to a large extent has replaced the use of leptazol and other convulsant drugs, since by its means the dose can be regulated more accurately, fits induced with more conformity, and the extremely unpleasant effects of the intravenous injection of large doses of leptazol and other drugs avoided. Revision of the section on radiology has resulted in the deletion of some general matter on the properties of radium, and in diagnostic radiology the new official nomenclature has been applied to the drugs used. Reference is made to the recent advances in the adoption of mass radiography of the chest, both for the services and the civilian population.

Bacteriological and Clinical Notes

The information in this section is, as in former editions, largely

complementary to the information concerning antitoxins, sera, vaccines, etc., given in Volume I. In addition, this section contains details of the ætiology and symptomatology of many parasitic diseases. The whole of the text has been carefully revised and in many instances entirely rewritten with a view to increasing its value to bacteriologists, physicians, pharmacists and others interested in the practical application of laboratory methods as aids to diagnosis. The particulars concerning the morphology, cultural characteristics, and biochemical reactions of the common pathogenic bacteria, have been amplified, particularly in the case of the salmonella group, the typhoid-paratyphoid group and the streptococci. Details of the composition of several new culture media, which assist the rapid isolation of bacteria, e.g., the dysentery and paratyphoid groups, have been added. The recognition of several outbreaks of trichiniasis in Great Britain in recent years seems to warrant the inclusion of an account of the clinical features of this disease, which it would seem may often pass undiagnosed if unsuspected. Notes have been added on the clinical features of cysticercosis in view of the increased prevalence of epilepsy from this cause among men returning from service in the East. The sections relating to beri-beri and pellagra have been omitted, since these diseases are now fully recognized as resulting from deficiency of vitamin B₁ and nicotine acid respectively. Likewise, there would seem to be no useful purpose in retaining details of the numerous theories of causation and obsolete forms of treatment of cancer.

The following are some additions of special interest:—

ABORTUS FEVER. Prophylactic inoculation of cattle with a vaccine made from an attenuated strain of *Br. abortus* is now sanctioned by the Ministry of Health; the use of a vaccine made from dead organisms has not proved satisfactory. Some notes are included on the diagnosis of abortus fever in the human subject, and should be read in relation to the general section on undulant fever, of which abortus fever is the commonest variety in Great Britain.

ACNE VULGARIS. The use of penicillin in glucose broth medium inhibits the growth of staphylococci and allows a primary growth of acne bacillus to be obtained in pure culture.

ACTINOMYCOSIS. Involvement of the central nervous system is a rare complication, and pathways of spread of the infection from a primary focus are discussed.

BACTERIAL FOOD POISONING. The notes on the Gaertner group of bacteria have been rewritten, and some details of methods for the differentiation of food poisoning organisms are included. Notes have been added on staphylococcus food poisoning due to an enterotoxin formed by some species of staphylococci, which have been implicated to an increasing extent in some recent outbreaks.

BLACKWATER FEVER. The ætiology of malarial hæmoglobinuria is considered to be allied to that of the hæmoglobinuria of primary

hæmolytic anæmia and may be due to the increased liberation of an intracellular hæmolysin.

BOTULISM. The exotoxins of *Clostridium botulinus* and methods for the detection of the toxin in blood and tissues are discussed.

COLIBACILLARY INFECTIONS. Methods for the differentiation of coliform organisms by the Voges and Proskauer reactions, the methyl red reaction, and the use of Koser's medium, are described.

DIPHTHERIA. The respective merits of the various media suggested for the isolation and identification of the *gravis* and *mitis* strains are compared.

DYSENTERY (BACILLARY). *B. dysenteriae* Schmitz is now recognized as a cause of bacillary dysentery, and the first outbreak in which this strain has been identified in Great Britain has been recorded. The media suggested by Wilson and Blair, and by Leifson, have greatly simplified the isolation of pathogenic bacteria from the intestinal contents.

GAS GANGRENE. The Nagler reaction has been suggested for rapidly identifying *Clostridium welchii* when present as a contaminant of war wounds. The relationship of the causal organism in welchii infections in humans with similar organisms infecting animals is discussed. *Vibrio Septique*, *Cl. œdematiens*, and *Cl. histolyticum* are other gas-forming anærobcs occasionally present in cases of human gas gangrene.

INFLUENZA. Since the isolation of influenza virus by Andrewes and his colleagues, a second type of virus has been recovered from influenza patients. The original virus (now termed virus "A") has not been present in some of the recent outbreaks, and it is possible that a serologically different virus (virus "B") may have been the ætiological agent. The method for the propagation of the virus on the developing chick embryo is described. The possibility of the existence of yet a third influenza virus, which the Americans have termed virus "Y," is discussed by Andrewes.

KALA-AZAR. The medium of Novy, MacNeal and Nicolle for the cultivation of leishmaniæ is used for blood culture in the diagnosis of this infection. The aldehyde test and the antimony test are useful aids to diagnosis but do not give positive results until one and two months respectively from the onset of the disease.

LEPROSY. An important development still under trial is the use of diphtheria antitoxin and diphtheria toxoid in the treatment of this disease.

LYMPHOPATHIA VENEREUM. This term has been adopted in place of the older designation, lympho-granuloma inguinale, in view of the recognition of the wide distribution of the lesions. Frei's antigen is used both for diagnosis and treatment, and may now be prepared from virus cultivated in the yolk sac of the chick embryo, as well as from infected mouse brain.

POLIOMYELITIS. The older view that the virus is transmitted by droplet infection and gains entry *via* the nasal mucosa and the olfactory tract has not satisfactorily explained many features of this disease, and there is now experimental and clinical evidence

to support the view that the most common route of infection is *via* the gastro-intestinal tract. Attempts to control the disease should be made as if it were an enteritis rather than influenza.

RICKETTSIAL DISEASES. The opportunity of this revision has been taken to group together the various rickettsial diseases, such as Japanese river fever, trench fever, Rocky Mountain spotted fever and *Fièvre Boutonneuse*, with the epidemic and non-epidemic group of typhus fevers. The control of these fevers provides a problem in war-time, when American and European troops are transported to endemic regions. Successful results have been reported from various methods of immunization against rickettsial infections, particularly from the use of chick embryo vaccine prepared by Cox's method.

SCARLATINA. Serological investigations have shown that in Great Britain the streptococci of scarlatina belong to Lancefield's group A, the commonest types being I, II, III and IV.

SPRUE. Attention has been directed to the resemblance of this condition to other states of "chronic jejuno-ileal insufficiency"; the similarity of the glossitis and stomatitis of sprue, and those of pellagra, pernicious anæmia, and idiopathic steatorrhœa, suggest a common ætiological factor in this disease-group.

STAPHYLOCOCCI. It has been suggested that staphylococci should be classified on the basis of the property of coagulase production. Some strains of *Staphylococcus aureus* and *Staphylococcus albus* produce a soluble exotoxin which is hæmolytic, dermonecrotic, and lethal to test animals.

STREPTOCOCCI. Study of Lancefield's groups in relation to their epidemiological significance has revealed the presence of streptococci other than group A in a number of human infections. Two serologically distinct varieties of hæmolysin, streptolysin O and streptolysin S have been obtained from group A hæmolytic streptococci. The use of gentian violet in blood agar permits the growth of streptococci whilst inhibiting staphylococci.

TRICHINIASIS. Several outbreaks in Great Britain have provided descriptions of the clinical and pathological features of this disease acquired by eating imperfectly cooked pork. Diagnosis may be assisted by a skin test or a precipitin test, using an antigen prepared from the trichinella larvæ.

TUBERCULOSIS. There is still a very prevalent opinion that bovine strains of the tubercle bacillus are less virulent for man than the human strains. Bacteriological surveys show that in Great Britain, and particularly in Scotland, the bovine strain is at least as virulent as the human form. Flotation methods for the separation of tubercle bacilli from sputum and the Lowenstein-Jensen medium for their isolation are described. A method for the cultivation of *M. tuberculosis* in hæmolysed blood enables cultures to be obtained in from 3 to 7 days.

TYPHOID FEVER. To be of value in diagnosis the Widal reaction must be applied in relation to the antigenic structure of the typhoid and paratyphoid bacilli. Methods for the preparation

of H and O agglutinating sera and antigens are described. The search for carriers among a series of suspected contacts is facilitated by tests for the detection of Vi antigen.

UNDULANT FEVER. The importance of blood cultures for diagnosis in the early stages and of agglutination tests in the later stages of the group of *Brucella* infections is stressed.

WHOOPIING COUGH. The production of an exotoxin from the *Hæmophilus pertussis* has been described; skin reactions and complement fixation tests have been suggested as alternatives to the cough plate method for diagnosis.

YELLOW FEVER. Considerable progress has been made in methods of prophylactic immunization, but a high incidence of jaundice after inoculation with prophylactic vaccine has been reported. Some new facts with regard to epidemiology have been brought to light as a result of the application of the mouse-protection test as an aid to diagnosis.

Acknowledgments

The fullest use has again been made of scientific papers and abstracts published in a wide range of chemical, pharmaceutical and medical periodicals, both British and foreign. The greatest care has been taken to select matter likely to be of most value to medical practitioners, pharmacists and analysts, and every effort made to ensure accuracy. If any errors are noted by readers or users of the book, the Editor will be grateful if his attention is drawn thereto and the Revision Committee will welcome suggestions regarding the subject matter or its arrangement by which the usefulness of *The Extra Pharmacopœia* may be increased.

Assistance has been obtained from several persons having special knowledge of different parts of the work and The Council of the Pharmaceutical Society desire to record their indebtedness to the staff of the Codex Department who have prepared this edition under the direction of the Codex Revision Committee and in particular to the following persons who have assisted them in the revision of certain sections of the book requiring expert knowledge on special subjects:—H. E. Archer, M.R.C.S., L.R.C.P., F.I.C., Ph.C.; H. F. Brewer, M.A., M.D.; Katherine H. Coward, D.Sc.; F. Hartley, Ph.D., B.Sc., B.Pharm., Ph.C.; W. M. Levitt, M.D., F.R.C.P.; Alistair MacGregor, M.D.; A. Maude; W. V. Mayneord, D.Sc.; T. McLachlan, F.I.C.; J. R. Nicholls, D.Sc., F.I.C.; R. Peck; A. H. T. Robb-Smith, M.B., B.S., M.R.C.S., L.R.C.P.; A. I. Robinson, Ph.C.; George Simon, M.D.; J. E. Woodhead, B.Sc., F.I.C., Ph.C., and P. H. Woodnoth, Ph.C.

June, 1943.

ABBREVIATIONS

The abbreviated titles of journals are those given in the *World List of Scientific Periodicals* (2nd Edn., 1934). When the reference is to a periodical of which two volumes are published during a year the number placed first indicates the first or second volume of the year followed by the year, and the last number refers to the page, thus, *Brit. med. J.*, i/1932, 250. When only one volume of a periodical is published each year, the reference gives the year and the page, thus, *Quart. J. Pharm.*, 1934, 341. In other cases the volume number is given in italics in addition to the year and page, thus, *J. biol. Chem.*, 1928, 77, 797.

α —optical rotation.

A.O.A.C.—Methods of Analysis of the Association of Official Agricultural Chemists, Washington.

A.P.F.—Australian Pharmaceutical Formulary.

A.R.—Reagent for Analytical Purposes.

Acta paediatr., *Stockh.*—Acta paediatrica.

Allen—Allen's Commercial Organic Analysis. 5th Edn., Vols. I-VI edited by S. S. Sadtler, E. C. Lathrop and C. A. Mitchell; Vols. VII-X edited by C. A. Mitchell (1924-1933).

Amer. J. Cancer—American Journal of Cancer.

Amer. J. Dis. Child.—American Journal of Diseases of Children.

Amer. J. Hyg.—American Journal of Hygiene.

Amer. J. med. Sci.—American Journal of Medical Sciences.

Amer. J. Obstet. Gynec.—American Journal of Obstetrics and Gynecology.

Amer. J. Pharm.—American Journal of Pharmacy.

Amer. J. Physiol.—American Journal of Physiology.

Amer. J. Publ. Hlth.—American Journal of Public Health.

Amer. J. Syph.—American Journal of Syphilis.

Amer. J. trop. Med.—American Journal of Tropical Medicine.

Amer. Perfum.—American Perfumer and Essential Oil Review.

Amer. Prof. Pharm.—American Professional Pharmacist.

Amer. Rev. Tuberc. (Suppl.)—American Review of Tuberculosis (Supplement).

Analyst—Analyst.

Ann. Eugen., *Camb.*—Annals of Eugenics.

Ann. Falsif.—Annales des Falsifications.

Ann. Hyg. publ., *Paris*—Annales d'hygiène publique et de médecine légale (industrielle et sociale).

Ann. Inst. Pasteur—Annales de l'Institut Pasteur.

Ann. Surg.—Annals of Surgery.

Ann. trop. Med. Parasit.—Annals of Tropical Medicine and Parasitology.

Apothekerztg., *Berl.*—Apothekerzeitung, Berlin.

Arch. Derm. Syph., *N.Y.*—Archives of Dermatology and Syphilology.

Arch. Dis. Childh.—Archives of Disease in Childhood.

Arch. exp. Path. Pharmacol.—Archiv für experimentelle Pathologie u. Pharmacologie.

Arch. int. Pharmacodyn.—Archives internationales de pharmacodynamie et de thérapie.

Arch. intern. Med.—Archives of Internal Medicine.

Arch. Kinderheilk.—Archiv für Kinderheilkunde.

Arch. klin. Chir.—Archiv für klinische Chirurgie.

Arch. Méd. Enf.—Archives de médecine des enfants.

Arch. Pharm., *Berl.*—Archiv der Pharmazie.

Arch. Pharm. Chemi.—Archiv für Pharmaci og Chemi.

Arch. Neurol. Psychiat., *Lond.*—Archives of Neurology and Psychiatry.

Arch. Radiol. Electrother.—Archives of Radiology and Electrotherapy.

Ass. méd.—Association médicale.

Aust. J. Pharm.—Australian Journal of Pharmacy.

B.H.P.—British Homeopathic Pharmacopoeia.

b.p.—boiling-point.

B.P.—British Pharmacopoeia, 1932.

B.P. '14—British Pharmacopoeia, 1914.

B.P. Add. I, II, III, IV, V, and VI.—First (1936), Second (1940), Third (1941), Fourth (1941), Fifth (1942), and Sixth (1943) Addenda to the British Pharmacopoeia, 1932.

- B.P.C.*—British Pharmaceutical Codex, 1934.
B.P.C. Supp.—Supplements to the British Pharmaceutical Codex, 1934.
B.V.H.—Bristol Voluntary Hospitals Pharmacopœia, 1935.
Ber. dtsch. chem. Ges.—Bericht der Deutschen Chemischen Gesellschaft.
Berl. klin. Wschr.—Berliner klinische Wochenschrift.
Biochem. J.—Biochemical Journal.
Biochem. Z.—Biochemische Zeitschrift.
Boll. Ist. sieroter., Milano—Bollettino dell'Istituto sieroterapico milanese.
Brit. chem. Abstr.—British Chemical Abstracts. (A) Pure Chemistry. (B) Applied Chemistry.
Brit. colon. Drugg.—British and Colonial Druggist (since 1915—British and Colonial Pharmacist).
Brit. colon. Pharm.—British and Colonial Pharmacist.
Brit. dent. J.—British Dental Journal.
Brit. J. Actino-Therap.—British Journal of Actinotherapy and Physiotherapy.
Brit. J. Biophys.—British Journal of Biophysics.
Brit. J. Child. Dis.—British Journal of Children's Diseases.
Brit. J. Derm.—British Journal of Dermatology.
Brit. J. exp. Path.—British Journal of Experimental Pathology.
Brit. J. phys. Med.—British Journal of Physical Medicine.
Brit. J. Radiol. (B.A.R.P. Sect.)—British Journal of Radiology (British Association for the Advancement of Radiology and Physiotherapy Section), continued since 1927 as British Journal of Radiology, New Series.
Brit. J. Radiol., N.S.—British Journal of Radiology, New Series.
Brit. J. Radiol. (Röntg. Soc. Sect.)—British Journal of Radiology (Röntgen Society Section), continued since 1927 as British Journal of Radiology, New Series.
Brit. J. Surg.—British Journal of Surgery.
Brit. J. vener. Dis.—British Journal of Venereal Diseases.
Brit. med. J.—British Medical Journal.
Brit. med. J. Epit.—British Medical Journal Epitome.
Brompton H.—Pharmacopœia of the Hospital for Consumption and Diseases of the Chest, 11th Edn., 1928.
Brooke—Tropical Medicine, Hygiene and Parasitology, by Gilbert E. Brooke, 1920.
Bruce and Dilling—Bruce and Dilling's Materia Medica and Therapeutics, by W. J. Dilling.
Bull. Acad. Méd., Paris—Bulletin de l'Académie de médecine.
Bull. Dep. Agric. Can.—Bulletin of the Department of Agriculture of the Dominion of Canada.
Bull. Fed. int. Pharm.—Bulletin de la Fédération internationale pharmaceutique.
Bull. Hlth Org. L. o. N.—Bulletin of the Health Organisation of the League of Nations.
Bull. Hyg.—Bulletin of Hygiene.
Bull. imp. Inst., Lond.—Bulletin of the Imperial Institute.
Bull. Inst. Pasteur—Bulletin de l'Institut Pasteur.
Bull. Off. int. Hyg. publ.—Bulletin mensuel de l'Office internationale d'hygiène publique.
Bull. Sci. Pharm.—Bulletin des Sciences pharmacologiques.
Bull. Soc. chim. Fr.—Bulletin, Société chimique de France.
Bull. Soc. méd. Hôp. Paris—Bulletin et mémoires de la Société médicale des hôpitaux de Paris.
Bull. tech. Mus., Sydney—Bulletin of the Technological Museum, Sydney.
C.H.W.—Formulæ of Chelsea Hospital for Women, 1927.
C.I.S.—Commission Internationale des Spécialités.
C.L.T.H.—Formulæ of the Central London Throat, Nose and Ear Hospital 3rd Edn., 1924.
C.X.H.—Charing Cross Hospital Pharmacopœia, 1935.
Canad. Form.—The Canadian Formulary, 1933.
Canad. med. Ass. J.—Canadian Medical Association Journal.
Canad. publ. Hlth J.—Canadian Public Health Journal.
Chem. Abstr.—Chemical Abstracts.
Chem. & Drugg.—Chemist and Druggist.
Chem. & Ind.—Chemistry and Industry, of the Society of Chemical Industry.
Chem. Ind. Rev.—Chemistry and Industry Review.
Chem. Weekbl.—Chemische Weekblad.

- Chem. Z.*—Chemische Zeitschrift.
Chem. Ztg.—Chemische Zeitung.
Chininum—Chininum Scriptiones Collectae, Bureau for increasing the use of Quinine, Amsterdam, 1925.
Clin. J.—Clinical Journal.
cm.—centimetre.
Colyer—Colyer's Dental Surgery and Pathology, by Sir J. F. Colyer, 6th Edn., 1931, and earlier issues (previously Smale and Colyer's Diseases and Injuries of Teeth).
C.R. Acad. Sci., Paris—Compte rendu hebdomadaire des séances de l'Académie des sciences.
C.R. Soc. Biol., Paris—Compte rendu hebdomadaire des séances et mémoires de la Société de biologie.
Cushny—Text-book of Pharmacology and Therapeutics, by A. R. Cushny, 10th Edn., revised by C. W. Edmunds and J. A. Gunn (1934).
[D]—Drugs or preparations coming within the scope of the Dangerous Drugs Acts, 1920 (as amended) and not exempt from control under the Dangerous Drugs Regulations, 1937.
Dansk Tidsskr. Farm.—Dansk Tidsskrift for Farmaci.
Dtsch. med. Wschr.—Deutsche medizinische Wochenschrift.
Disp.—Art of Dispensing, published by *The Chemist and Druggist*, London, 10th Edn., 1926.
Dixon—Manual of Pharmacology, by the late W. E. Dixon, F.R.S., 7th Edn., 1929.
D.S.I.R.—Dept. of Scientific and Industrial Research Leaflets—H.M.S.O.
D.T.F.—Drug Tariff Formulary issued by the Ministry of Health.
E.G.A.—Pharmacopœia of the Elizabeth Garrett-Anderson Hospital, 1926.
Ec. Prod. India—Economic Products of India.
Edinb. med. J.—Edinburgh Medical Journal.
Emery—Clinical Bacteriology and Hæmatology, by W. d'Este Emery, 6th Ed., 1921.
Endocrinology—Endocrinology.
F.E. VIII—Farmacopea Española. Octava Edición, 1930.
f.p.—freezing-point.
Fr. Cx.—Codex Medicamentarius Gallicus, Pharmacopée Française (1937).
Finnemore—Essential Oils, their Chemistry and Technology, by H. Finnemore, 1926.
g.—gramme.
G.H.—Pharmacopœia of Guy's Hospital, 1937.
Gehe—Gehe's Codex, 6th Edn., 1933.
Ghosh—Treatise on Materia Medica and Therapeutics, by the late R. Ghosh, I.M.S. Edited by B. H. Deane, 12th Edn., 1930.
Glasg. med. J.—Glasgow Medical Journal.
gr.—grain.
Gradwohl and Blaivas—The Newer Methods of Blood and Urine Chemistry by R. B. H. Gradwohl and A. J. Blaivas, 2nd Edn., 1920.
Gt. Orm. H.—Pharmacopœia of the Hospital for Sick Children, Great Ormond Street, 1933.
Hager—Handbuch der Pharmaceutischen Praxis, revised by G. Fredericks, G. Arends and H. Zörnig, 1925.
Hale-White—Hale-White's Materia Medica, Pharmacy, Pharmacology and Therapeutics, revised by A. H. Douthwaite, 24th Edn., 1939.
Hare—Text-Book of Practical Therapeutics, by H. A. Hare, 21st Edn., 1930.
Harper Adams Util. Poult. J.—Harper Adams Utility Poultry Journal.
Harrison—Chemical Methods in Clinical Medicine, by G. A. Harrison, 2nd Edn., 1937.
Hawk—Practical Physiological Chemistry, by P. B. Hawk and O. Bergeim, 11th Edn., 1938.
Helv. chim. Acta—Helvetica chimica acta.
Hewlett and McIntosh—A Manual of Bacteriology, 9th Edn., revised by R. T. Hewlett and J. McIntosh, 1932.
Hoppe-Seyl. Z.—Hoppe-Seyler's Zeitschrift für physiologische Chemie.
Hospitalstidende—Hospitalstidende.
Hutchison—Food and Principles of Dietetics, by R. Hutchison and V. H. Mottram, 7th Edn., 1933.
I.A.—International Agreement, 1930.

- I.H.*—Pharmacopœia of the Infants' Hospital, Vincent Square, London, 1939.
I.V.—iodine value.
I. c. Add.—Indian and Colonial Addendum (1900) to the B.P. 1898.
Indian J. med. Res.—Indian Journal of Medical Research.
Indian med. Gaz.—Indian Medical Gazette.
Indian med. Res. Mem.—Indian Medical Research Memoirs.
Industr. Engng Chem. (anal. Edn.)—Industrial and Engineering Chemistry (Analytical Edition).
Int. Conf. trop. Amer.—Proceedings of the International Conference on Health Problems in Tropical America, 1924, United Fruit Co., Boston.
Int. J. Leprosy—International Journal of Leprosy.
Int. J. Med.—International Journal of Medicine and Surgery, now included in Surgical Journal.
J. R. Army med. Cps.—Journal of the Royal Army Medical Corps.
J. R. nav. med. Serv.—Journal of the Royal Naval Medical Service.
J. agric. Sci.—Journal of Agricultural Science.
J. Allergy—Journal of Allergy.
J. Amer. chem. Soc.—Journal of the American Chemical Society.
J. Amer. diet. Ass.—Journal of the American Dietetic Association.
J. Amer. med. Ass.—Journal of the American Medical Association.
J. Amer. pharm. Ass., pharm. Abstr.—Pharmaceutical abstracts published in the Journal of the American Pharmaceutical Association.
J. Amer. pharm. Ass., pract. Pharm. Edn.—Journal of the American Pharmaceutical Association, Practical Pharmacy Edition.
J. Amer. pharm. Ass., Sci. Edn.—Journal of the American Pharmaceutical Association, Scientific Edition.
J. Ass. off. agric. Chem., Wash.—Journal of the Association of Official Agricultural Chemists.
J. biol. Chem.—Journal of Biological Chemistry.
J. Cancer Res.—Journal of Cancer Research.
J. chem. Soc.—Journal of the Chemical Society.
J. chem. Soc. Abstr.—Journal of the Chemical Society Abstracts (continued since 1926 as British Chemical Abstracts).
J. clin. Invest.—Journal of Clinical Investigation.
J. clin. Res.—Journal of Clinical Research.
J. comp. Path.—Journal of Comparative Pathology and Therapeutics.
J. Dairy Res.—Journal of Dairy Research.
J. Dairy Sci.—Journal of Dairy Science.
J. exp. Med.—Journal of Experimental Medicine.
J. Franklin Inst.—Journal of the Franklin Institute.
J. gen. Physiol.—Journal of General Physiology.
J. Hyg., Camb.—Journal of Hygiene.
J. Immunol.—Journal of Immunology.
J. Indian med. Ass.—Journal of the Indian Medical Association.
J. infect. Dis.—Journal of Infectious Diseases.
J. Instn. elect. Engngs.—Journal of the Institution of Electrical Engineers.
J. Lab. clin. Med.—Journal of Laboratory and Clinical Medicine.
J. Laryng.—Journal of Laryngology (Rhinology) and Otology.
J. ment. Sci.—Journal of Mental Science.
J. Obstet. Gynaec.—Journal of Obstetrics and Gynaecology of the British Empire.
J. Path. Bact.—Journal of Pathology and Bacteriology.
J. Pediat.—Journal of Pediatrics.
J. Pharm. Chim., Paris—Journal de pharmacie et de chimie.
J. Pharmacol.—Journal of Pharmacology and Experimental Therapeutics.
J. Physiol.—Journal of Physiology.
J. Prat., Paris—Journal des praticiens.
J. Röntgen Soc.—Journal of the Röntgen Society, continued from 1924 to 1927 as The British Journal of Radiology (Röntgen Society Section), and since 1927 as The British Journal of Radiology, New Series.
J. State Med.—Journal of State Medicine.
J. Soc. chem. Ind., Lond.—Journal of the Society of Chemical Industry.
J. Suisse Pharm.—Journal suisse de pharmacie, now Schweizerische Apothekerzeitung.
J. Text. Inst., Manchr.—Journal of the Textile Institute, Manchester.
J. trop. Med. (Hyg.)—Journal of Tropical Medicine and Hygiene.

- K.C.H.*—King's College Hospital Pharmacopœia, 1934.
Kenwood—Public Health Laboratory Work, by H. R. Kenwood, 8th Edn., 1925.
Klin. Wschr.—Klinische Wochenschrift.
Knox—Radiography and Radio-Therapeutics, by Robert Knox, 4th Edn., in 2 vols. (Vol. II completed and edited by W. M. Levitt), 1923-32.
L.C.C.—London County Council Pharmacopœia, 1936.
L.H.—London Hospital Pharmacopœia, 1934.
L.L.—London Lock Hospitals Pharmacopœia.
L.S.H.—London Skin Hospital.
Lancet—Lancet.
Leprosy Rev.—Leprosy Review.
m.—minim.
m.a.—milliampere.
m.p.—melting-point.
M.R.C.—Medical Research Council.
M.R.I.—Manchester Royal Infirmary Pharmacopœia, 1933.
Mackie and MacCartney—Handbook of Practical Bacteriology, 6th Edn., 1942.
May—Chemistry of Synthetic Drugs, by Percy May, 3rd Edn., 1921.
Med. Annu.—Medical Annual.
Med. J. Aust.—Medical Journal of Australia.
Med. J. Rec.—Medical Journal and Record.
Med. Klinik—Medizinische Klinik.
Med. Offr—Medical Officer.
Med. Pr.—The Medical Press and Circular.
Med. Rec., N.Y.—Medical Record.
Medicine, Baltimore—Medicine, Baltimore.
Mem. Univ. Calif.—Memoirs of the University of California.
Merck—E. Merck's Annual Report of recent advances in Pharmaceutical Chemistry and Therapeutics.
Merck's Index—Merck's Index, 5th Edn., 1940.
Mfg. Chem.—Manufacturing Chemist.
mg.—milligramme.
Mid. H.—Middlesex Hospital Pharmacopœia, 1933.
Mikrochemie—Mikrochemie.
Milit. Surg.—Military Surgeon.
ml.—millilitre.
Mod. Tech. in Treatment—Modern Technique in Treatment, Vols. 1-4, 1925-28, *The Lancet*, London.
M Schr. Geburtsh. Gynäk.—Monatsschrift für Geburtshilfe u. Gynäkologie.
Muir and Ritchie—Manual of Bacteriology, by R. Muir and J. Ritchie, 10th Edn., 1937.
Münch. med. Wschr.—Münchener medizinische Wochenschrift.
Murrell—What to do in Cases of Poisoning, by William Murrell, 13th Edn., 1925, revised by P. Hamill.
n.—refractive index.
N.F. VII—National Formulary of Unofficial Preparations, issued by the American Pharmaceutical Association, 7th Edn., 1942.
N.H.—Prescription Formulæ for Use in Naval Hospitals, 1930.
N.I.F.—National Formulary for National Health Insurance Purposes, issued by the British Medical Association, 3rd Edn., 1939.
N.N.R.—New and Non-official Remedies, issued annually by the American Medical Association.
N.W.F.—National War Formulary.
Nature, Lond.—Nature, London.
Naturwissenschaften—Naturwissenschaften.
Nav. med. Bull., Wash.—Naval Medical Bulletin, Washington.
New Engl. J. Med.—New England Journal of Medicine.
Norsk farm. Tidsskr.—Norsk farmaceutisk Tidsskrift.
Nutr. Abstr. Rev.—Nutrition Abstracts and Reviews.
N.Y. St. J. Med.—New York State Journal of Medicine.
[P1]—Part 1 of the Poisons List.
[P2]—Part 2 of the Poisons List.
P.E.H.C.—Pharmacopœia of the Princess Elizabeth of York Hospital for Children (formerly the East London Hospital), 1933.
P.G. VI.—German Pharmacopœia, 1926.
P.J.F.—Pharmaceutical Journal Formulary.

- P.L.*—Pharmacopœia Londinensis, 1851.
P.M.C.E.—Select Parliamentary Committee on Proprietary Medicines Enquiry 1912-13.
P.M.H.—Pharmacopœia for use in Military Hospitals (*H.M.S.O.*).
P. Argent. II.—Pharmacopœia of the Argentine Republic, 2nd Edn., 1919.
P. Austr.—Austrian Pharmacopœia, 1906.
P. Belg. IV.—Belgian Pharmacopœia, 1930.
P. Dan.—Danish Pharmacopœia, 1933.
P. Helv. V.—Swiss Pharmacopœia, 1933.
P. Ital. V.—Italian Pharmacopœia, 1929.
P. Jap. V.—Japanese Pharmacopœia, 1935.
P. Ned. V.—Netherlands Pharmacopœia, 1926, and Supplements I and II.
P. Russ.—Russian Pharmacopœia, 1934.
P. Svec.—Swedish Pharmacopœia, 1925.
Paris méd.—Paris médical. La semaine du clinicien.
Perfum. essent. Oil Rec.—Perfumery and Essential Oil Record.
Ph. Form.—Pharmaceutical Formulas, 9th Edn., Second Reprint, 1921, by Peter MacEwan; and 10th Edn., Vol. I, 1929, revised by S. W. Woolley and G. P. Forrester, Vol. II, 1934, revised by G. P. Forrester, *The Chemist and Druggist*, London.
Pharm. Acta Helvet.—Pharmaceutica Acta Helvetiæ.
Pharm. J.—Pharmaceutical Journal.
Pharm. Weekbl.—Pharmaceutisch Weekblad voor Nederland.
Pharm. Zentralk.—Pharmazeutische Zentrallhalle f. Deutschland.
Pharm. Ztg. Berl.—Pharmazeutische Zeitung.
Philipp. J. Sci.—Philippine Journal of Science.
Physiol. Rev.—Physiological Reviews.
Pr. méd.—Presse médicale.
Practitioner—Practitioner.
Prescriber—Prescriber.
Proc. Mayo Clin.—Proceedings of Staff Meetings of the Mayo Clinic.
Proc. nat. Acad. Sci.—Proceedings of the National Academy of Science.
Proc. roy. Soc.—Proceedings of the Royal Society.
Proc. roy. Soc. Edinb.—Proceedings of the Royal Society of Edinburgh.
Proc. R. Soc. Med.—Proceedings of the Royal Society of Medicine.
Proc. Soc. exp. Biol., N.Y.—Proceedings of the Society for Experimental Biology and Medicine.
Publ. Hlth. Lond.—Public Health.
Publ. Hlth Rep., Wash.—Public Health Reports, issued by the United States Public Health Service.
Quart. Bull. Hlth Org. L. o. N.—Quarterly Bulletin of the Health Organisation of the League of Nations.
Quart. J. exp. Physiol.—Quarterly Journal of Experimental Physiology.
Quart. J. Med.—Quarterly Journal of Medicine.
Quart. J. Pharm.—Quarterly Journal of Pharmacy and Pharmacology.
Quart. J. Physiol.—Quarterly Journal of Experimental Physiology.
R.D.H.—Pharmacopœia of the Royal Dental Hospital, London, 1926.
R.F.H.—Pharmacopœia of the Royal Free Hospital, London, 1934.
R.I.—Refractive Index.
R.I. Edinb.—Pharmacopœia of the Royal Infirmary, Edinburgh, 1935.
R.L.O.H.—Pharmacopœia of the Royal London Ophthalmic Hospital (Moorfields Eye Hospital), 1933.
R.N.H.—Pharmacopœia of the Royal Northern Group of Hospitals, 1938.
R.V.I.—Pharmacopœia of the Royal Victoria Infirmary, Newcastle-on-Tyne.
Rem.—Remington's Practice of Pharmacy, 7th Edn., 1926.
Rep. Brit. Emp. Cancer Campgn—Report of the British Empire Cancer Campaign.
Rep. Cancer Res. Fd.—Report of the Imperial Cancer Research Fund.
Rep. Inst. med. Res. F.M.S.—Report of the Institute for Medical Research, Federated Malay States.
Rep. med. Offr Minist. Hlth, Lond.—Report of the Chief Medical Officer, the Ministry of Health.
Rep. med. Res. Coun., Lond.—Report of the Medical Research Council.
Rep. metrop. Asylums Bd.—Report of the Metropolitan Asylums Board.
Rep. metrop. Wat. Bd.—Report of the Metropolitan Water Board.

- Rep. publ. Hlth med. Subj., Lond.*—Report on Public Health and Medical Subjects, Ministry of Health.
- Retail Chem.*—Retail Chemist.
- [S1]—First Schedule to the Poisons Rules, 1935. Other Schedules are indicated by the corresponding numerical suffix.
- S.R.A., F.D., No. 2, Rev. 4.*—Service and Regulatory Announcements, Food and Drug No. 2 (Fourth Revision); issued by the United States Department of Agriculture, Food and Drug Administration, Nov., 1936.
- S.R. & O.*—Statutory Rules and Orders, His Majesty's Stationery Office, London.
- S.V.*—saponification value.
- St. B. H.*—Pharmacopœia of St. Bartholomew's Hospital, 1936.
- St. G. H.*—Pharmacopœia of St. George's Hospital, 1927.
- St. J. H.*—Pharmacopœia of St. John's Hospital for Skin Diseases, 1934.
- St. M. H.*—Pharmacopœia of St. Mary's Hospital, 1934.
- St. Mark's H.*—Pharmacopœia of St. Mark's Hospital for Diseases of the Rectum and Colon, 1935.
- St. T. H.*—Pharmacopœia of St. Thomas' Hospital, 1935.
- S. Afr. med. J.*—South African Medical Journal.
- Schmidt*—Ausführliches Lehrbuch der Pharmaceutischen Chemie, Vol. I (Inorganic), Vol. II (Organic), Part I (1922), Part II (1923), by Ernst Schmidt.
- Schweiz. ApothZtg*—Schweizerische Apothekerzeitung.
- Schweiz. med. Wschr.*—Schweizerische medizinische Wochenschrift.
- Sci. Rep. Cancer Res. Bd, Lond.*—Scientific Reports on the Investigations of the Imperial Cancer Research Fund.
- Science*—Science.
- Secret Remedies*—Secret Remedies, What they Cost and What they Contain.—British Medical Association (1909); also "More Secret Remedies" (1912).
- Seidell*—Solubilities of Inorganic and Organic Substances, 2nd Edn., 1920.
- Soddy*—Interpretation of Radium, by F. Soddy, 4th Edn., 1920.
- Sp. gr.*—specific gravity.
- Spec. Rep. Food Invest. Bd, Lond.*—Special Report, Food Investigation Board, Department of Scientific and Industrial Research.
- Spec. Rep. Ser. med. Res. Comm.*—National Health Insurance, Medical Research Committee, Special Report Series.
- Spec. Rep. Ser. med. Res. Coun., Lond.*—Special Report Series, Medical Research Council, London.
- Stitt*—Practical Bacteriology, Hæmatology and Animal Parasitology, by E. R. Stitt, P. W. Clough and M. C. Clough, 9th Edn., 1938.
- Surg. Gynec. Obstet.*—Surgery, Gynecology and Obstetrics.
- Svensk farm. Tidskr.*—Svensk farmaceutisk Tidskrift.
- T.H.*—Pharmacopœia of the Golden Square Throat, Nose and Ear Hospital, 1935.
- Thorpe*—Dictionary of Applied Chemistry.
- Topley and Wilson*—The Principles of Bacteriology and Immunity, by W. W. C. Topley and G. S. Wilson, 1929.
- Trans. Brit. Soc. dent. Surg.*—Transactions of the British Society of Dental Surgeons.
- Trans. R. Soc. trop. Med. Hyg.*—Transactions of the Royal Society of Tropical Medicine and Hygiene.
- Trop. Dis. Bull.*—Tropical Diseases Bulletin.
- U.C.H.*—Pharmacopœia of University College Hospital, 1933.
- U.F.C. '25*—Fourteenth Annual Report, United Fruit Co., Medical Dept., 1925.
- U.S.D.*—United States Dispensatory, 21st Edn., 1937.
- U.S.P. XII*—Pharmacopœia of the United States, 1942.
- Urol. cutan. Rev.*—Urologic and Cutaneous Review.
- V.H.C.*—Pharmacopœia of the Victoria Hospital for Children (Chelsea), 1920.
- v/v*—volume in volume.
- v/w*—volume in weight.
- Vet. J.*—Veterinary Journal.
- Vic. Park*—City of London Hospital for Diseases of the Heart and Lungs, Victoria Park, E.2, 1926.
- W.*—Pharmacopœia of the Hospital for Women (Soho Square), 1927.
- W.O. Memo. Med. Dis.*, 1941—War Office Memorandum on Medical Diseases in Tropical and Subtropical Areas, 1941 (H.M.S.O.).
- w/v*—weight in volume; *w/w*—weight in weight.
- W.H.*—Pharmacopœia of Westminster Hospital, 1934.

Ward and Smith—Recent Advances in Radium, by W. Roy Ward and A. J. Durden Smith, 1933.

Wenyon—Protozoology, by C. M. Wenyon, 2 vols., 1926.

Whitla—Whitla's Dictionary of Treatment, 8th Edn., 1938.

Wien. klin. Wschr.—Wiener klinische Wochenschrift.

Wynter Blyth—Foods: Their Composition and Analysis, by the late A. Wynter Blyth and M. Wynter Blyth, 7th Edn., revised by H. E. Cox, 1927.

Yearb. Pharm.—The Yearbook of Pharmacy (since 1928 The Quarterly Journal of Pharmacy and Pharmacology).

Z. anal. Chem.—Zeitschrift für analytische Chemie.

Z. angew. Chem.—Zeitschrift für angewandte Chemie und Zentralblatt für technische Chemie. (Since 1932, continued as *Angewandte Chemie*.)

Z. ges. exp. Med.—Zeitschrift für die gesamte experimentelle Medizin.

Z. Hyg. Infekt.Kr.—Zeitschrift für Hygiene und Infektionskrankheiten.

Z. Immunforsch.—Zeitschrift für Immunitätsforschung und experimentelle Therapie.

Z. klin. med.—Zeitschrift für klinische Medizin.

Z. phys. Chem.—Zeitschrift für physikalische Chemie.

Z. physiol. Chem.—Zeitschrift für physiologische Chemie.

Z. Untersuch. Lebensmitt.—Zeitschrift für Untersuchung der Lebensmittel.

Zbl. Bakt.—Zentralblatt für Bakteriologie.

INTERNATIONAL ATOMIC WEIGHTS

| Element | Sym- bol | Atomic Weight | Element | Sym- bol | Atomic Weight |
|---------------|-------------|------------------|-----------------|-------------|------------------|
| Aluminium .. | Al | 26.97 | Molybdenum .. | Mo | 95.95 |
| Antimony .. | Sb | 121.76 | Neodymium .. | Nd | 144.27 |
| Argon .. | A | 39.944 | Neon .. | Ne | 20.183 |
| Arsenic .. | As | 74.91 | Nickel .. | Ni | 58.69 |
| Barium .. | Ba | 137.36 | Nitrogen .. | N | 14.008 |
| Beryllium .. | Be | 9.02 | Osmium .. | Os | 190.2 |
| Bismuth .. | Bi | 209.00 | Oxygen .. | O | 16.0000 |
| Boron .. | B | 10.82 | Palladium .. | Pd | 106.7 |
| Bromine .. | Br | 79.916 | Phosphorus .. | P | 30.98 |
| Cadmium .. | Cd | 112.41 | Platinum .. | Pt | 195.23 |
| Calcium .. | Ca | 40.08 | Potassium .. | K | 39.096 |
| Carbon .. | C | 12.010 | Praseodymium | Pr | 140.92 |
| Cerium .. | Ce | 140.13 | Protoactinium.. | Pa | 231 |
| Cesium .. | Cs | 132.91 | Radium .. | Ra | 226.05 |
| Chlorine .. | Cl | 35.457 | Radon .. | Rn | 222 |
| Chromium .. | Cr | 52.01 | Rhenium .. | Re | 186.31 |
| Cobalt .. | Co | 58.94 | Rhodium .. | Rh | 102.91 |
| Columbium .. | Cb | 92.91 | Rubidium .. | Rb | 85.48 |
| Copper .. | Cu | 63.57 | Ruthenium .. | Ru | 101.7 |
| Dysprosium .. | Dy | 162.46 | Samarium .. | Sm | 150.43 |
| Erbium .. | Er | 167.2 | Scandium .. | Sc | 45.10 |
| Europium .. | Eu | 152.0 | Selenium .. | Se | 78.96 |
| Fluorine .. | F | 19.00 | Silicon .. | Si | 28.06 |
| Gadolinium .. | Gd | 156.9 | Silver .. | Ag | 107.880 |
| Gallium .. | Ga | 69.72 | Sodium .. | Na | 22.997 |
| Germanium .. | Ge | 72.60 | Strontium .. | Sr | 87.63 |
| Gold .. | Au | 197.2 | Sulphur .. | S | 32.06 |
| Hafnium .. | Hf | 178.6 | Tantalum .. | Ta | 180.88 |
| Helium .. | He | 4.003 | Tellurium .. | Te | 127.61 |
| Holmium .. | Ho | 164.94 | Terbium .. | Tb | 159.2 |
| Hydrogen .. | H | 1.0080 | Thallium .. | Tl | 204.39 |
| Indium .. | In | 114.76 | Thorium .. | Th | 232.12 |
| Iodine .. | I | 126.92 | Thulium .. | Tm | 169.4 |
| Iridium .. | Ir | 193.1 | Tin .. | Sn | 118.70 |
| Iron .. | Fe | 55.85 | Titanium .. | Ti | 47.90 |
| Krypton .. | Kr | 83.7 | Tungsten .. | W | 183.92 |
| Lanthanum .. | La | 138.92 | Uranium .. | U | 238.07 |
| Lead .. | Pb | 207.21 | Vanadium .. | V | 50.95 |
| Lithium .. | Li | 6.940 | Xenon .. | Xe | 131.3 |
| Lutecium .. | Lu | 174.99 | Ytterbium .. | Yb | 173.04 |
| Magnesium .. | Mg | 24.32 | Yttrium .. | Y | 88.92 |
| Manganese .. | Mn | 54.93 | Zinc .. | Zn | 65.38 |
| Mercury .. | Hg | 200.61 | Zirconium .. | Zr | 91.22 |

The Editor would welcome any suggestions regarding the subject matter or arrangement of the work, from Medical Men, Pharmacists, or Analysts.

ANALYTICAL ADDENDA TO CHEMICALS AND MATERIA MEDICA IN VOLUME I.

ACACIA

Acacia (B.P.). Occurs in rounded or ovoid tears and the powder does not comply with the official description. Loses not more than 15% of its weight at 100° and should yield not more than 5% of ash. (All *B.P.* ash values are calculated with reference to the drug dried at 100°.) It should be free from tannins, starch and dextrins and the *B.P.* requires it to be almost entirely soluble in an equal volume of water, but the *U.S.P. XII* permits not more than 1% of water-insoluble residue, limits the ash to 4% and acid-insoluble ash to 0.5% and permits not more than 15% of moisture. *Gomme Arabique, Fr. Cx. 1937*, has a specific rotation in 5% aqueous solution of about -26° .

The best varieties are Kordofan, Mogadore and Senegal gums, but dark-coloured gums are not suitable for medicinal purposes.

Tentative methods for the identification of gums are described in *Methods of Analysis (A.O.A.C., 1940, 596)*. Acacia, tragacanth, starch, quince, Irish moss, agar, karaya and galagum are distinguished by tests with chlorzinciodide, ruthenium red, methylene blue, etc.

Acidity of Gum Acacia. The amount of sodium hydroxide required to neutralise 1000 g. of gum varies from 2.48 to 3.2 g., giving an average of 2.84 g. The clarity of the solution is in no way related to its reaction.

Identification of Common Gums. Acacia, tragacanth, agar, Irish moss, quince, and ghatti gum distinguished by Millon's reagent, lead acetate, potassium hydroxide, tannic acid, etc. *A useful table.—Pharm. J., ii/1931, 46.*

Detection of Karaya, Tragacanth and Agar. One drop of the preparation suitably diluted on a slide is treated with a mixture of zinc iodide and potassium iodide solutions. Karaya particles are coloured green. The particles are seen as irregular billowy masses which are resilient when pressed under a cover slip. Tragacanth is similar in appearance but the masses are more uniform. It can be identified by the characteristic striations. Agar (e.g., in mayonnaise) may be detected by shaking about 50 g. with twice its volume of alcohol, filtering and defatting the residue with ether. The residue is dried and suspended in 50 ml. of cold water to remove soluble gums, dextrin and sugars, and the insoluble product heated rapidly to boiling with 25 ml. of water to remove protein. The liquid is filtered while hot and the filtrate digested with 5 ml. of malt extract, if starch is present, a further 5 ml. being added after 5 minutes digestion. The liquid is concentrated until it gels on cooling and is then tested on a slide with iodine solution. Agar gives a distinct red colour. A method is also given for detecting agar in the presence of other mucilages and of gelatin.—*J. D. Wildman, J. Ass. off. agric. Chem., Wash., 1935, 637.*

ACETANILIDUM

Acetanilidum (B.P.C.). $C_8H_9ON = 135.1$. M.p., 113° to 115° . Ash, not more than 0.1%. Complies with tests for absence of phenacetin and for neutrality. Acetanilide is not included in *B.P.*, but is official in *U.S.P. XII*, *P. Helv. V*, etc. The *Fr. Cx. 1937* includes a limit of water-soluble substances of 0.2%.

Determination. Hydrolyse 1.5 g. by boiling for 15 minutes with 50 ml. of 20% hydrochloric acid and dilute to 500 ml. To 25 ml. of this solution add

excess standard potassium bromide-bromate solution to precipitate tribromoaniline, and estimate excess bromine in the usual way.—*J. chem. Soc. Abstr.*, i/1921, 604.

Tabellæ Acetanilidi (N.F. VII). Contain 92.5 to 107.5% of the labelled amount of acetanilide, including all tolerances. Assayed by extracting a weighed quantity of powdered tablets with chloroform, removal of the chloroform and titrating the residue, dissolved in water and hydrochloric acid, with bromide-bromate solution previously standardised against a pure sample of acetanilide.

Methods for the determination of mixtures of acetanilide with phenacetin, with caffeine, with caffeine and codeine, with caffeine and quinine, with caffeine, quinine and morphine and with sodium salicylate are described in *Methods of Analysis (A.O.A.C., 1940, 559, et seq.)*.

Methylacetanilide. The detection and identification of N-methylacetanilide in mixtures of antipyretics is described.—A. Denoel, per *Analyst*, 1939, 831

ACIDUM ACETICUM

Acidum Aceticum (B.P.). Contains from 32.5 to 33.5% *w/w* of $\text{CH}_3\cdot\text{COOH}$; sp. gr., 1.044 to 1.045. The diluted acid, Acidum Aceticum Dilutum, now contains 6% *w/w*, whereas the corresponding acid of the B.P. '14 contained only 5%. Acidum Aceticum Glaciale (B.P.) contains not less than 99% by weight with a f.p. of not less than 14.8° and a sp. gr. of 1.055 to 1.058.

Glacial acetic acid of the U.S.P. XII contains 99.4% of $\text{CH}_3\cdot\text{COOH}$ and Acidum Aceticum (U.S.P. XII) contains from 36 to 37% by weight of $\text{CH}_3\cdot\text{COOH}$; the glacial acid should have a congealing point not below 15.6° . Acide Acétique, *Fr. Cx.* 1937, contains not less than 98% *w/w* of $\text{CH}_3\cdot\text{COOH}$. The dilute acid is prepared by diluting the 98% acid with 9 parts of water. Acidum aceticum, *P.G. VI*, contains not less than 96% and Acidum aceticum dilutum, *P.G. VI*, from 29.7 to 30.6% of $\text{CH}_3\cdot\text{COOH}$. Acidum aceticum concentratum and Acidum aceticum dilutum, *P. Helv. V*, contain 98 to 100% and 29.5 to 30.5% *w/w* respectively of $\text{CH}_3\cdot\text{COOH}$.

A British Standard Specification (B.S.S. No. 576—1934) has been issued by the British Standards Institution for Glacial Acetic Acid and Dilute Acetic Acids. The specification for Glacial Acetic Acid, 99 to 100%, includes description, acetic acid content, crystallising-point (not lower than 14.8°), residue on evaporation, iron, chlorides, sulphates, heavy metals, substances reducing permanganate, formic acid, aldehydes and ketonic substances, and sampling, and the appendices describe the methods and apparatus to be used. The specification for Dilute Acetic Acids deals with Acetic Acids, 80%, 60%, and 40%. The iron which may be present in these substances is determined colorimetrically by means of thioglycolic acid in the presence of ammonia. B.S.S. No. 578—1934 refers to Technical Acetic Acid, 98 to 100%, and to Technical Acids, 80%, 60%, and 40%.

Potassii Acetas (B.P.). $\text{CH}_3\cdot\text{COOK} = 98.12$. Loses not more than 5% of its weight at 100° and the dried salt contains not less than 99% of $\text{CH}_3\cdot\text{COOK}$ when assayed by ignition and subsequent titration of the alkaline residue with acid. The U.S.P. XII salt is of the same strength after drying to constant weight at 150° . Acétate de Potassium, *Fr. Cx.* 1937, contains 95% of the pure substance, as indicated by the ignited potassium sulphate.

Sodii Acetas (B.P.C.). $\text{CH}_3\cdot\text{COONa}, 3\text{H}_2\text{O} = 136.1$. The crystalline salt should contain the equivalent of 99.5 to 105%

of the pure salt. Sodii Acetas, *N.F. VII*, loses from 36 to 41% of water at 120° and then contains not less than 99% of the pure anhydrous salt.

Thallii Acetas (*B.P.C.*). $\text{CH}_3\cdot\text{COOTl}=263\cdot4$. Assayed gravimetrically by precipitating the base as thallos iodide, and contains not less than 98%.

Zinci Acetas (*B.P.C.*). $(\text{CH}_3\cdot\text{COO})_2\text{Zn}, 2\text{H}_2\text{O}=219\cdot5$. Contains not less than 99·5% of the pure acetate when assayed on its zinc content by precipitation with mercuric ammonium thiocyanate solution and subsequent titration with iodate solution, as described in the Pharmacopœia for Zinci Sulphas. Zinci Acetas, *U.S.P. XII*, contains from 83·16 to 87·32% of $(\text{CH}_3\cdot\text{COO})_2\text{Zn}$, corresponding to not less than 99·5% of the hydrated salt; it is assayed by precipitation as carbonate and weighing as oxide.

Liquor Ammonii Acetatis Dilutus (*B.P.*). Determined by titration of the acetic acid liberated upon fixation of the ammonia with solution of formaldehyde, with *N*/1 sodium hydroxide, using phenolphthalein indicator, contains 6·9 to 7·5% *w/v* of $\text{C}_2\text{H}_7\text{O}_2\text{N}$. Liquor Ammonii Acetatis, *N.F. VII*, contains 6·5 to 7·5% *w/v* of $\text{C}_2\text{H}_7\text{O}_2\text{N}$.

Liquor Ammonii Acetatis Fortis (*B.P.*) contains 55 to 60% *w/v* of $\text{C}_2\text{H}_7\text{O}_2\text{N}$.

Acidum Aminoaceticum (*U.S.P. XII*). $\text{C}_2\text{H}_5\text{O}_2\text{N}=75\cdot07$. Loses not more than 0·2% at 100° and then contains 18·4 to 18·8% of *N*, corresponding to 98·5% of $\text{C}_2\text{H}_5\text{O}_2\text{N}$, when assayed by the Kjeldahl method. Ash, not more than 0·1%.

Acidum Trichloroaceticum (*B.P.*). $\text{CCl}_3\cdot\text{COOH}=163\cdot4$. Assayed by ignition with sodium carbonate and titration of the chloride formed with silver nitrate and ammonium thiocyanate and contains not less than 98% of the pure acid. The *U.S.P. XII* acid, dried to constant weight over sulphuric acid, contains not less than 99% and is assayed by titration with normal sodium hydroxide solution. The *P. Helv. V* requires a content of 99·64% of $\text{CCl}_3\cdot\text{COOH}$, and the acid should have a solidifying point of 55° to 59°.

Acetonum (*B.P.*). Sp. gr. at 15·5°, 0·796 to 0·801. At least 95% by volume distils between 56° and 58° when measured under standard conditions. Tests for alkalinity, acidity, oxidisable substances, moisture, and residue on evaporation are also prescribed. The *U.S.P. XII* prescribes an iodometric method for the assay of acetone and requires it to contain not less than 99% by weight of $\text{CH}_3\cdot\text{CO}\cdot\text{CH}_3$. Acétone, *Fr. Cx. 1937*, by iodometric assay contains not less than 98% of the pure substance.

Diacetone Alcohol. A British Standard Specification for Diacetone Alcohol, 4-hydroxy-4-methyl-pentanone-2 (*B.S.S. No. 549—1934*), includes requirements regarding description, specific gravity, distillation range, flash-point, miscibility with water, water, acidity, and sampling.

Amyl Acetate. A British Standard Specification (*B.S.S. No. 552—1934*) for Amyl Acetate includes requirements regarding description, specific gravity, distillation range, residue on evaporation, water, acidity, ester content, and sampling.

Æthylis Acetas (B.P.C.), determined by treatment with N/1 sodium hydroxide during 30 mins. and back titration with standard acid to phenolphthalein, contains not less than 90% w/w of $C_4H_8O_2$. **Æthylis Acetas, N.F. VII**, contains not less than 99% of $C_4H_8O_2$; in assaying, the sample is refluxed on a water-bath for one hour with the standard alkali.

A specification for Ethyl Acetate (B.S.S. No. 553—1934) includes requirements regarding description, specific gravity, distillation range, residue on evaporation, water, acidity, ester content (not less than 96%), and sampling.

Normal Butyl Acetate. B.S.S. No. 551—1934 for Normal Butyl Acetate (acetic ester of primary normal butyl alcohol) includes requirements regarding description, specific gravity, distillation range, residue on evaporation, acidity, ester content, and sampling. The appendices describe the methods and apparatus to be used.

Carbacholum (B.P. Add. III). $C_6H_5O_2N_2Cl = 182.6$. Loses not more than 0.5% at 110° , and then contains not less than 99.5% of the pure substance, by titration with N/10 silver nitrate to potassium chromate indicator. M.p. (with decomposition), 210° to 212° . Residue on incineration, not more than 0.1%.

ACIDUM ACETYSALICYLICUM

Acidum Acetylsalicylicum (B.P.). Assayed by hydrolysis with excess of N/2 sodium hydroxide and titration with acid with a blank experiment under the same conditions. It contains not less than 99.5% of the pure compound, has a m.p. of 135° to 138° and a limit test for free salicylic acid by which the proportion does not exceed about 0.05%.

The m.p. of the U.S.P. XII product is not below 135° and the test for free salicylic acid is equivalent to about 0.1%. **Acidum acetylsalicylicum, P. Helv. V**, is determined by titrating a solution in alcohol to phenolphthalein with N/10 sodium hydroxide and contains from 99.5 to 100% of the pure substance.

Tabellæ Acidi Acetylsalicylici (U.S.P. XII). Contain 95 to 105% of the labelled amount of $C_9H_8O_4$, including all tolerances. Assayed by suspending a weighed quantity of the powdered tablets in neutral alcohol and titration with N/10 sodium hydroxide using phenolphthalein indicator, then adding a known excess of N/10 sodium hydroxide to the titration flask and heating on a boiling water bath for 15 minutes, and finally cooling and titrating against N/10 sulphuric acid. A blank titration is performed.

An official method for the determination of acetylsalicylic acid in tablets containing phenacetin and caffeine is described in *Methods of Analysis (A.O.A.C., 1940, 569)*. 0.2 g. of the powdered tablets with water are completely extracted with chloroform and the chloroform evaporated to about 2 ml. 10 ml. of sulphuric acid (1+9) is added, and the mixture digested, the flask partly in a boiling water-bath and connected to a reflux condenser, for 30 mins. The caffeine and salicylic acid are then extracted from the mixture with chloroform, the salicylic acid transferred to 5% sodium carbonate solution and the solution, after evaporating any chloroform, diluted to 100 ml. with water. 25 to 40 ml. of 0.2N iodine is then added and digested for 1 hour on a steam-bath, the free iodine removed with a few drops of sodium thiosulphate solution, the precipitate collected on a Gooch, washed, dried at 100° and weighed. The weight of tetraiodophenylenequinone ($C_6H_2I_4O_2$) $\times 0.4016$ = total salicylic acid. Deduct any free salicylic acid present. $\text{Salicylic acid} \times 1.304$ = acetylsalicylic acid.

Assay of Aspirin, Phenacetin and Caffeine tablets. Determine the acetylsalicylic acid by dissolving about 0.5 g. of powdered sample in alcohol, titrating with N/10 sodium hydroxide, adding excess of alkali, refluxing and back-titrating with acid. The phenacetin and caffeine are determined together

by dissolving in water with a little alkali, and shaking out with chloroform. The extracted material is refluxed with dilute sulphuric acid and the caffeine shaken out with chloroform and weighed. The acid solution remaining is neutralised with sodium bicarbonate. After the addition of a few drops of acetic anhydride, the mixture is shaken out with chloroform. This operation is repeated four times, the chloroform solution is evaporated to dryness and the phenacetin weighed.—P. Sorgdrager, *Pharm. Tijdscher. Ned. Indie*, 1940, 17, 227.

Determination of Free Salicylic Acid in Acetylsalicylic Acid. Dissolve salicylic acid, 1 g., in alcohol, 60 ml., and adjust to 100 ml. with water. 10 ml. of this solution may then be diluted to 1000 ml. for the standard, making 1 ml. = 0.0001 g. acid. Dissolve 0.6 g. of the sample to be tested in 9 ml. of alcohol, dilute with water to 90 ml. and mix well. Take two exactly similar Nessler glasses. Into one pour 60 ml. of the solution, into the other the remaining 30 ml., together with 3 ml. of alcohol, and adjust to the volume of the first. This gives a difference of 0.2 g. of acetylsalicylic acid in similar mixtures of alcohol and water. One ml. of a 1% solution of iron alum is added to each, mixed, and the colour matched by adding the salicylic acid solution.—A. J. Jones, *Chem. & Drugg.*, 1919, 402.

Official methods for the determination of free and total salicylic acid in acetylsalicylic acid are described in *Methods of Analysis (A.O.A.C., 1940, 567)*.

A solution yields a buff-coloured precipitate with ferric chloride until hydrolysed by the addition of a little hydrochloric acid, which yields the typical violet colour of salicylate (developing particularly on warming).

The ferric chloride test for free salicylic acid in acetylsalicylic acid is insufficient to prevent adulteration, etc., in that the addition of borax, sodium phosphate, tartaric acid, citric acid and other oxy-acids will readily prevent or mask the colour ordinarily produced with ferric chloride.

Tartaric Acid added to aspirin tablets would mask the ferric iron test if salicylic acid were present. 1% of citric acid will mask the presence of 0.2% of free salicylic acid.—A. Nutter Smith, *Chem. & Drugg.*, ii/1930, 1630.

Determination of Free Acetic Acid. A. Nutter Smith (*Yearb. Pharm.*, 1920, 421) described a process by which the sample (1 g.) previously finely powdered is spread on muslin and the acetic acid evolved is aspirated into 50 or 100 ml. of distilled water for $\frac{1}{2}$ or 1 hour. The liquid is then titrated with N/500 caustic soda. Each ml. of this represents 0.00012 g. of acetic acid.

The decomposition of acetylsalicylic acid in aqueous solution in the presence of alkali citrate or acetate is independent of the concentration of the salt or of the acid. Solutions (at room temperature) decompose about 10% during the first day and 50% in a week. The rate of decomposition increases very rapidly with rise of temperature.—C. Morton, *Quart. J. Pharm.*, 1933, 495.

Calcii Acetylsalicylas (B.P.C.).

$(\text{CH}_3\text{CO}\cdot\text{OC}_6\text{H}_4\cdot\text{COO})_2\text{Ca}\cdot 2\text{H}_2\text{O} = 434.2$. Contains not less than 95% when assayed by weighing the sulphate formed on ignition with sulphuric acid.

ACIDUM BENZOICUM

Acidum Benzoicum (B.P.). $\text{C}_6\text{H}_5\cdot\text{COOH} = 122.0$. The natural and synthetic acids are official; at least 99.5% of free acid must be present and a limit test for chlorinated compounds is prescribed. M.p., 121° to 122° . The U.S.P. XII acid, after drying at 100° for 2 hours, contains not less than 99.3%; the solution in dilute alcohol neutralised to phenolphthalein is titrated with standard sodium hydroxide and a correction is applied for the proportion of chlorinated compounds present. *Acide Benzoique*, *Fr. Cx.* 1937, melts at 121° and contains not less than 99% of the pure substance. Both *Acidum benzoicum* and *Acidum benzoicum e resina* are official in the *P. Helv. V*; the former melts at 120.5° to 121.5° and the latter between 118° and 121° .

Detection in Foodstuffs. Extract with a mixture of ether and petroleum ether in equal parts; this evaporated may contain saccharin (taste), salicylic acid (by its colour with ferric chloride), and benzoic acid—recognised by odour, crystalline form, and conversion into aniline blue by heating with rosaniline and aniline.

Colorimetric Estimation in Cordials, Etc. The aniline blue reaction is unsatisfactory, as acetic, succinic and salicylic acids also give the reaction. Best results with a modification of Halphen's Reaction, hydroxylamine hydrochloride being employed as reducing agent. Presence of benzoic acid indicated by fine red colour.—A. J. Jones, *Yearb. Pharm.*, 1925, 493.

Caution needed in search for traces of preservatives in caramel and boiled sugar sweets, which yield a crystalline acid substance (m.p. 122°), giving a violet colour with ferric chloride, similar to benzoic acid.—per *Yearb. Pharm.*, 1927, 185.

Determination in Foodstuffs (Fruits and Vegetables), in permitted amounts, specified under the Regulations in force since Jan. 1, 1927. A lengthy process, starting with steam distillation after saturating if necessary with salt, and subliming in presence of sand for 1 to 1½ hours at 160°, and ultimately weighing the benzoic acid present.—G. W. Monier-Williams, *Rep. publ. Hlth med. Subj.*, No. 39, 1927, per *Yearb. Pharm.*, 1927, 182.

In the Monier-Williams method it is quite clear that unless the oven used for sublimation is arranged to agree in all respects with the one described, either a higher temperature or a longer time may be required for quantitative recovery of the benzoic acid.—R. W. Sutton and O. Hitchen, *Analyst*, 1940, 502.

The application of Grossfield's modification of Mohler's test for benzoic acid to the detection and determination in foodstuffs is described. Details are given of the results obtained on various foods and the percentage errors are recorded for 31 different articles.—E. T. Illing, *Analyst*, 1932, 224.

Various methods of estimation of benzoates and salicylates.—E. B. R. Prideaux and A. O. Bentley, *Pharm. J.*, 1/1923, 427.

A distillation method of determination in foods (butter, margarine, and egg products) and in wines (not sweet).—per *Yearb. Pharm.*, 1927, 183. Also an adaptation of the French official method for detection in wines, showing 1 mg. in 100 ml. of wine.—*ibid.*, 1925, 149.

Official methods for detection and estimation of benzoic acid in foods are described in *Methods of Analysis (A.O.A.C.)*, 1940, 456).

The following method, a modification of Nicholls's method (*Analyst*, 1928, 19), was found to be more satisfactory when dealing with food products. Reagents required:—(A) *Iron solution for oxidation*.—A solution of 2.7 g. of anhydrous ferric chloride in 13 ml. of N/1 sulphuric acid made up to 100 ml. with water. (B) *Iron solution for colorimetric determination*.—0.1 g. of anhydrous ferric chloride in 20 ml. of N/1 hydrochloric acid diluted to 100 ml. with water. (This solution eliminates interference due to *p*-hydroxybenzoic acid and other compounds.) (C) 0.2% hydrogen peroxide solution prepared by diluting 2 ml. of 20 vol. solution to 60 ml. with water. The benzoic acid is isolated by extraction with an immiscible solvent or by steam distillation from an acid solution saturated with salt. The acid is neutralised with dilute ammonia, the excess of ammonia removed by boiling until the vapours are neutral to litmus, and the solution made up to a definite volume. To an aliquot portion, containing not more than 5 mg. of benzoic acid, is added 5 ml. of N/10 sulphuric acid and water to 15 ml., followed by 1 ml. of iron solution A and 1 ml. of 0.2% peroxide solution, the mixture being well mixed and then heated in a boiling water-bath for 15 minutes. The product is treated with about 5 g. of ammonium sulphate and the salicylic acid extracted with three successive quantities, each of 15 ml., of ether, each ethereal extract being washed with 5 ml. of water. The ether is distilled off at 50°, the residue dissolved in 50 ml. of alcohol 10%, and the solution treated with 1 ml. of iron solution B. The colour produced is compared with that given by a standard solution of salicylic acid in 10% alcohol. The weight of benzoic acid originally present may be found from the weight of salicylic acid produced by means of the following table:—

| | | | | | | | | |
|--|------|------|-----|------|-----|------|------|------|
| Salicylic acid formed, mg. | 0.1 | 0.15 | 0.2 | 0.25 | 0.3 | 0.35 | 0.4 | 0.45 |
| Benzoic acid originally present, mg. . . | 0.77 | 1.25 | 1.8 | 2.3 | 2.8 | 3.3 | 3.85 | 5.0 |

The method is specific so far as is known, with the exception of saccharin, and if this is present the separated benzoic acid must be extracted with carbon tetrachloride in which saccharin is insoluble.—F. W. Edwards, H. R. Nanji and M. K. Hassan, *Analyst*, 1937, 172.

Ammonii Benzoas (B.P.C.). $C_6H_5 \cdot COONH_4 = 139.1$. Contains not less than 98% and is assayed by adding excess of standard acid, separating the benzoic acid by means of ether and titrating the excess of acid with sodium hydroxide using phenol red as indicator.

Benzylis Benzoas (B.P. Add. IV). $C_6H_5 \cdot COOCH_2 \cdot C_6H_5 = 212.1$. The *B.P.* method for esters in essential oils with 2 hours boiling over a flame is used and a percentage of not less than 99 is required. Sp. gr., 1.121 to 1.125; freezing point not below 18.5° . $n_{D^{20}}$, 1.568 to 1.570. Ash, not more than 0.05%. Benzylis Succinas, *B.P.C.*, is required to have a purity of 99% and is assayed by the same method.

Sodii Benzoas (B.P.). $C_6H_5 \cdot COONa = 144.0$. Moisture at 110° not more than 4% and the dried salt contains not less than 99% of the pure benzoate. The method of assay of the *B.P.* 1914, in which the alkaline residue obtained on ignition was titrated, is replaced by the ether-acid process in which the alkali of a neutralised solution is titrated with standard sulphuric acid to bromophenol blue after removing most of the liberated benzoic acid with ether. Sodii Benzoas, *U.S.P. XII*, after drying at 110° , contains not less than 99%, assayed by titration of an ethereal mixture with $N/2$ hydrochloric acid, using methyl orange indicator. The *Fr. Cx.* 1937 substance loses not more than 3% at 100° ; contains not less than 97% of the pure substance.

Ammonii Hippuras (B.P.C.). $C_6H_5 \cdot CO \cdot NH \cdot CH_2 \cdot COONH_4 = 196.1$. This ammonium salt is readily soluble and should not contain any appreciable amount of free hippuric acid. When assayed for nitrogen by the Kjeldahl method it must contain the equivalent of not less than 98% of the pure hippurate.

Acidum Cinnamicum (B.P.C.). $C_6H_5 \cdot CH : CH \cdot COOH = 148.1$. Determined in the same way as benzoic acid, it is required to contain not less than 99%. Benzoic acid is tested for by titrating the filtrate obtained after shaking 1 g. with 100 ml. of boiled and cooled water at 20° for 1 hour, and must not exceed about 4%.

Coumarinum (B.P.C.). $C_9H_6O_2 = 146.1$. Melts between 68° and 70° , with limit of 0.05% for ash and test for absence of acetanilide and readily carbonisable matter.

Vanillinum (B.P.C.). 4-Hydroxy-3-methoxybenzaldehyde, $CH_3O \cdot C_6H_3(OH) \cdot CHO = 152.1$. Melts between 80° and 82° , with limit of 0.05% for ash and a carbylamine test on 0.1 g. for absence of acetanilide. It is official in the *U.S.P. XII* and the standard of purity is the same as that of the *B.P.C.* Vanilla, *N.F. VII*, yields not less than 12% of anhydrous dilute alcohol extractive.

Official methods for the determination of vanillin and coumarin in flavouring agents are described in *Methods of Analysis (A.O.A.C., 1940, 320)*. 50 ml. of an extract is diluted to 80 ml. and evaporated to 50 ml., repeating this operation, 25 ml. of lead acetate (8%) solution added, adjusted to 100 ml., shaken, stood for 18 hours and filtered. 50 ml. of the filtrate is extracted with ether, the ether washed with ammonia water (1 + 11), the ammoniacal solutions acidified with

hydrochloric acid (1 + 2) and extracted with ether. After evaporating the ether, the residue of vanillin is weighed and should melt at about 80°. For coumarin, the first ether extract is evaporated and weighed. The residue should melt at 67° and on addition of a few drops of N/10 iodine to 0.5 ml. of hot solution a brown precipitate, gathering into green flecks, should be produced. The colorimetric method for vanillin consists of clearing a solution of 8 to 12 mg. with 4 ml. of lead solution (5% each neutral and basic lead acetate), diluting to 100 ml. and filtering. To 5 ml. of filtrate and 5 ml. of standard vanillin solution (0.1 mg. per ml.) 5 ml. of phosphotungstic-phosphomolybdic acid reagent is added respectively and diluted with saturated sodium carbonate solution to 50 ml. After filtering the solutions the blue colours produced are compared.

"Ethyl" Vanillin (*m*-ethoxy-*p*-hydroxybenzaldehyde) has chemical properties very closely resembling those of vanillin and is four or five times stronger in flavour. It can be determined by a bromine absorption method.—H. C. Lockwood, *Analyst*, 1934, 730.

Differentiation from Vanillin: Dissolve in hydrochloric acid, cool, add hydrogen peroxide, shake, and stand until precipitation complete. Add an equal volume of benzene, shake. The blue precipitate of vanillin remains in the aqueous layer, whereas that of the "ethyl" vanillin dissolves in the benzene to give a violet solution.—P. Stadler and K. Wagner, *Z. anal. Chem.*, 1938, *III*, 391–393.

Determination of Coumarin in Presence of Vanillin. Coumarin can be separated from vanillin in extracts of vanilla or other plant extracts by steam distillation in the presence of potassium sulphate (0.5 g. for 10 to 12.5 ml. of extract). The extract is diluted with 100 ml. of water and the distillation is conducted with dry steam, the pressure being reduced to 140 mm., until the contents of the distillation flask are evaporated to dryness. 100 ml. of water is then added and the distillation repeated. The condenser is rinsed down and the distillate diluted to 1000 ml. To 20 ml. in a 50 ml. graduated flask is added 5 ml. of 2% sodium carbonate solution, the liquid is then heated in a boiling water-bath for 5 to 10 minutes and cooled. 5 ml. of cold diazonium solution is added and the mixture diluted to volume. After standing 15 minutes, colorimetric comparison is made with standards containing known amounts of coumarin and treated similarly. The diazonium solution is prepared as follows: 3.5 g. of *p*-nitroaniline is dissolved in 45 ml. of concentrated hydrochloric acid and the solution diluted to 500 ml. This solution and a 5% *w/v* solution of sodium nitrite are stored in a refrigerator at 0° to 3°. 5 ml. of each solution is mixed in a 100 ml. flask, cooled in the refrigerator for 5 minutes, then 10 ml. of nitrite solution added, and the mixture cooled again and finally diluted to 100 ml. with ice-cold water. The resulting solution is cooled for 15 minutes before use.—J. Duncan and R. B. Dustman, *Industr. Engng Chem. (anal. Edn.)*, 1937, 416.

Determination of β -Ionone in Flavouring Substances. A solution of the flavouring substance containing 1 to 10 mg. of β -ionone in 5 ml. of alcohol is treated with 90 to 95 mg. of solid *m*-nitrobenz-hydrazide and the mixture is warmed (without loss of alcohol) on a steam bath. Warm water (10 ml.) is added, and if the solution becomes cloudy it is warmed until clear. It is then treated with 0.2 ml. of glacial acetic acid and the lightly stoppered flask is allowed to cool slowly. If 2 mg. or more of β -ionone are present, crystals begin to form within 30 minutes after the mixture has attained room temperature. The crystallising liquid is allowed to stand for at least two hours and is then placed in a refrigerator overnight or for a period not exceeding 48 hours, and is filtered through a sintered glass crucible with the aid of suction. The crystals are washed with 15 ml. of cold 30% alcohol and dried in a vacuum oven at 70°. The weight of β -ionone is found by multiplying the weight of the precipitate by 0.541.—J. B. Wilson, *J. Ass. off. agric. Chem., Wash.*, 1939, 22, 378.

ACIDUM BORICUM

Acidum Boricum (B.P.). H_3BO_3 = 61.84. Contains not less than 99.5%, determined by titration with sodium hydroxide with the addition of glycerin, and phenol violet as indicator; it complies with a test for solubility in boiling alcohol. It is official

in the *U.S.P. XII*, and after drying over sulphuric acid, should contain not less than 99.5% of the pure substance, using phenolphthalein as indicator and a 50% concentration of glycerin being used in the titration.

Detection of Boric Acid. See also *Milk Analysis*, p. 795.

Moisten congo red paper with a saturated aqueous solution and dry over a small flame; a blue colour is gradually developed. By using 1 in 1000 solution of congo red in a capsule and adding a little of a boric acid solution the presence of 0.00001 g. may be detected. The test is best applied to methyl or ethyl boric ester obtained by distillation.—per *Yearb. Pharm.*, 1925, 138.

The solution suspected to contain a borate is made slightly alkaline with sodium hydroxide and evaporated practically to dryness. The residue is treated with 1 ml. of concentrated sulphuric acid and cooled. 2 ml. of methyl alcohol is added and the mixture is transferred to a test tube fitted with a rubber stopper and two glass tubes. One of these extending to the bottom is bent at right angles and acts as a mouthpiece. The other conducts the vapours and is also bent, but drawn to a long capillary at least 3 cm. long and not more than 0.5 mm. bore. When air is blown through the apparatus the bubbles rising through the heated solution convey volatile methyl borate (if present), which tints a small bunsen flame a characteristic green colour.—A. Gabriel and H. G. Tanner, *J. Amer. chem. Soc.*, per *Chem. & Drugg.*, ii/1928, 777.

Sensitive Reaction. A 0.05% solution of carmine in strong sulphuric acid changes from red to blue in the presence of boric acid.—F. P. Zorbin, per *J. Amer. pharm. Ass.*, 1938, 353.

Official qualitative and quantitative methods for the determination of boric acid and borates in foods are described in *Methods of Analysis (A.O.A.C., 1940, 459)*.

Determination in Foodstuffs. 40 to 50 g. of sample is moistened with 10 ml. of 2N sodium hydroxide, the water evaporated on a steam-bath and the sample ashed. The ash is transferred to a Kjeldahl flask using the minimum quantity of water, the dish is rinsed with dilute sulphuric acid, and the ash in the flask dissolved in a further quantity of the acid by warming. Methyl red is added, followed by 30% sodium hydroxide until the liquid is alkaline, and the contents of the flask are then evaporated over a Bunsen flame, with continuous agitation, to 1 to 2 ml. After cooling, 60 ml. of methyl alcohol and 1 ml. of methyl red solution are added, followed by concentrated sulphuric acid to render strongly acid. The Kjeldahl flask is closed with a cork through which pass two tubes. One leads into a vertical double surface condenser, the exit tube of which is elongated and dips beneath the surface of alkaline methyl alcohol in a 400 ml. conical flask. The other passes to the bottom of the Kjeldahl flask and connects directly with the conical flask, its exterior portion being lagged by enclosure in a rubber tube. Immediately above the stopper of the conical flask is a side-tube sealed on to the condenser outlet; the side-tube runs up close to the condenser and carries a splash-bulb and funnel. The conical flask contains 0.5 ml. of N/1 sodium hydroxide and a few drops of phenolphthalein solution. The Kjeldahl flask is attached to the apparatus at an angle of 30° and is heated. When sufficient methyl alcohol has collected in the conical flask, the latter is also strongly heated so that the vapour bubbles through the liquid in the Kjeldahl flask. After 30 minutes the Kjeldahl flask is cooled in a beaker of cold water, the conical flask is removed and the condenser tube is washed into it with water. The remaining methyl alcohol is boiled off, the solution acidified to methyl red with N/10 sulphuric acid, boiled to remove carbon dioxide and readjusted when cold with N/20 sodium hydroxide until just not acid, more phenolphthalein added and then the solution is titrated to the phenolphthalein end-point in the presence of 1 g. of mannitol. A blank determination is also made on water. 1 ml. of N/20 sodium hydroxide corresponds to 0.0031 g. of boric acid.—R. S. Alcock, *Analyst*, 1937, 522.

Carbasus Acidi Borici (B.P.C. Supp. IV). Assayed by the process of the *B.P.C.* Shake in a stoppered bottle, a weighed quantity of the gauze (equivalent to about a quarter square yard) with 50 parts of boiled water and 40 parts of glycerin; cool and titrate with N/1 sodium hydroxide to phenolphthalein or phenol violet; from 4 to 6% of H_2BO_3 should be indicated.

Gossypium Acidi Borici (B.P.C. Supp. IV). Treated in the same manner as *Carbasus Acidi Borici* it contains from 4 to 6% of H_2BO_3 .

Linteum Acidi Borici (*B.P.C. Supp. IV*). Determined as for Carbasus Acidi Borici, contains 4 to 6% of H_2BO_3 . A piece of lint (measuring 3 inches by 3 inches) becomes saturated within 12 seconds when placed lightly, unraised side downwards, on the surface of water at 20° .

Borax (*B.P.*). $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O} = 381.4$. Assayed by the same method as boric acid, after neutralisation to methyl orange with sulphuric acid, it contains not less than 99% or more than the equivalent of 103% of $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$. *Sodii Boras, U.S.P. XII*, contains 52.32 to 54.92% of the anhydrous salt corresponding to not less than 99% of the crystallised substance, determined by titration with N/2 hydrochloric acid, using methyl red indicator. *Borate de Sodium Officinal, Fr. Cx. 1937*, by titration in glycerin and water with N/1 sodium hydroxide to phenolphthalein, contains not less than 99% of the pure substance. 1 ml. N/1 sodium hydroxide = 0.1907 g. Borax, *P.G. VI*, contains 52.3 to 54.3% of anhydrous sodium tetraborate, $\text{Na}_2\text{B}_4\text{O}_7$.

Sodii Perboras (*B.P.C.*). $\text{NaBO}_3 \cdot 4\text{H}_2\text{O} = 153.9$. Estimated by means of its oxidising action on potassium iodide in acidified solution and titration of the liberated iodine with sodium thio-sulphate, it contains from 96% to the equivalent of 103% of $\text{NaBO}_3 \cdot 4\text{H}_2\text{O}$. *Sodii Perboras, U.S.P. XII*, by titration with potassium permanganate, contains 9% of available oxygen, corresponding to about 86.5% of $\text{NaBO}_3 \cdot 4\text{H}_2\text{O}$. The substance of the *Fr. Cx. 1937*, by titration with N/10 potassium permanganate, contains not less than 86.6% of the pure salt corresponding to 9% of active oxygen.

ACIDUM CITRICUM

Acidum Citricum (*B.P.*) $\text{C}_3\text{H}_4(\text{OH})(\text{COOH})_3 \cdot \text{H}_2\text{O} = 210.1$. Assayed by titration with sodium hydroxide, using thymol blue as indicator, it should contain from 99.5% to the equivalent of 101% of the pure substance; limits of tartaric and oxalic acids are included. The *U.S.P. XII* requires a standard of purity of 97% but uses phenolphthalein as indicator for titration; 0.5 g. with 5 ml. of sulphuric acid kept at 90° for 1 hour, should be no darker than a specified control fluid.

A method for the determination of citrate by oxidation with potassium permanganate in the presence of mercuric sulphate in which formates, tartrates, etc., do not interfere.—W. F. Bruce, *Ind. Engng Chem. (anal. Edn.)*, 1934, 283.

Detection of Citric and Tartaric Acids, etc., in Presence of Sugar. The charring due to the presence of only a trace of sugar is sufficient to mask the colour produced in the resorcinol and sulphuric acid test for tartaric acid. The acids may be separated from sugar by shaking with methylated ether in which they are sufficiently soluble to yield a positive reaction when the test is applied to the residue obtained on evaporating the solution. 5 g. of sample is shaken for about 1 minute with 10 ml. of the ether and then filtered. The ether is evaporated and 1 ml. of water added. 2 to 3 drops of the solution are tested for tartaric acid with resorcinol and sulphuric acid. The remainder of the solution is tested for citric acid with mercuric sulphate and potassium permanganate. If a negative or only a slight positive reaction for tartrate is obtained, the same amount of sample should be tested for cream of tartar by shaking with acidified ether (1 drop of dilute sulphuric acid to 20 ml. of ether) and proceeding as before.—R. de Giacomi, *Analyst* 1937, 731.

Determination of Citric Acid in Mixtures containing also Sucrose and Tartaric Acid. A dilute solution of the citric acid is treated with sulphuric acid and potassium bromide solution; after addition of 5% potassium permanganate solution until a brown precipitate persists, followed, after one hour, by ferrous sulphate in 1% sulphuric acid (20%), the mixture is kept at 0° overnight; the filtered and washed precipitate is then dried in a vacuum desiccator, dissolved out with spirit and ether and the dried crucible again weighed. The difference in weight = pentabromacetone, a correction being made for the volume of filtrate and washings.—Lampitt and Rooke, *Analyst*, 1936, 654.

The method of Lampitt and Rooke does not give good results in the presence of sucrose, maltose or any other readily oxidised material. Modifications are given rendering the process more suitable for milk and milk products.—P. S. Arup, *Analyst*, 1938, 635.

Liquor Magnesii Citratis (U.S.P. XII). Contains magnesium citrate equivalent to 1.6 to 1.9% *w/v* of MgO , determined by precipitation and ignition to $Mg_2P_2O_7$.

Mangani Citras Solubilis (N.F. VII). Contains 48 to 52% of $(C_6H_5O_7)_2Mn_2$. Determined by precipitation with ammonia and hydrogen peroxide, followed by ignition of the precipitate to manganous-manganic oxide.

Potassii Citras (B.P. Add. I). $C_6H_5O_7K_3 \cdot H_2O = 324.4$. By titration of the ash with standard sulphuric acid it contains not less than 99% of $C_6H_5O_7K_3 \cdot H_2O$. Limit tests for tartrate and oxalate are included. 2 g. boiled with 25 ml. of water and cooled requires for neutralisation to thymol blue, not more than 0.5 ml. of N/10 sulphuric acid or of N/10 sodium hydroxide. The U.S.P. XII salt is assayed similarly and after drying to constant weight at 180° contains 99%. Loss at 180°, 3 to 6%.

Sodii Citras (B.P. Add. I). $C_6H_5O_7Na_3 \cdot 2H_2O = 294.1$. Assayed by the same method as the potassium salt it is required to contain 99% of the pure substance. Complies with the test for alkalinity or acidity described under Potassii Citras. The U.S.P. XII requires the same purity after drying at 150°; moisture loss at 150° 10 to 13%. The *Fr. Cx.* 1937, by weight as sulphate, requires a purity of 99%. Natrium citricum tribasicum, *P. Helv. V*, is the crystalline salt containing $5\frac{1}{2}$ molecules of water of crystallisation; by ignition and titration of the alkaline residue it should yield the equivalent of not less than 98% of $C_6H_5O_7Na_3 \cdot 5\frac{1}{2}H_2O$.

ACIDUM FORMICUM

Acidum Formicum (B.P.C.). Contains not less than 24% and not more than 26% *w/w* of $H \cdot COOH$ when assayed by digestion with alkaline potassium permanganate, the excess permanganate being titrated in acid solution with oxalic acid and a blank experiment made. Tests for the absence of acrolein and allyl formate and for limits of non-volatile matter and acetic acid are prescribed. Acidum Formicum, *N.F. VII*, is of the same strength, when the total acidity is titrated with sodium hydroxide. Acidum formicum, *P.G. VI*, contains 24 to 25% of $H \cdot COOH$. Acidum formicum, *P. Helv. V*, contains 24 to 25% *w/w* of $H \cdot COOH$.

An official quantitative method for the determination of formic acid in foods is described in *Methods of Analysis (A.O.A.C., 1940, 466)*.

Determination of formic acid in foodstuffs.—J. Grossfeld and R. Payfer, per *Analyst*, 1940, 168.

Calcii Formas (B.P.C.). $(\text{H}\cdot\text{COO})_2\text{Ca}=130\cdot1$. Determined by the same method as formic acid it should contain not less than 98% of the pure substance.

Potassii Formas (B.P.C.). $\text{H}\cdot\text{COOK}=84\cdot10$. Estimated for formate content, equivalent to 95% of $\text{H}\cdot\text{COOK}$.

Sodii Formas (B.P.C. Supp. IV). $\text{H}\cdot\text{COONa}=68\cdot2$. Assayed by the method for calcium formate, it contains not less than 96% of the pure substance.

ACIDUM GLYCEROPHOSPHORICUM

Acidum Glycerophosphoricum (B.P.C.). Contains 19 to 21% *w/w* of $\text{C}_3\text{H}_5\text{O}_4\text{P}$; limit of combined alkali (as Na_2O), 0·5%; limit of free phosphate (as P_2O_5), 0·5%. Assayed by the *B.P.C.* '34 process by titration with normal sodium hydroxide to bromocresol green and titration with sodium hydroxide to thymol blue, followed by boiling with neutralised calcium chloride solution and further titration to thymol blue, the latter indicating free phosphoric acid present; the difference between the first thymol blue titration and twice the bromocresol green indicates the combined alkali present, and the first thymol blue titration less the free phosphate and the bromocresol green titration represents the glycerophosphoric acid, calculated on the neutralisation of one H ion. Free glycerin is determined by separating the glycerophosphate as calcium salt from alcoholic solution, evaporating and drying the filtrate, and should not exceed 2%.

The analysis of glycerophosphate syrups.—G. Middleton, *Yearb. Pharm.*, 1926, 421.

The alkali and alkaline earth salts of glycerophosphoric acid on the English market are for the most part perfectly definite salts and of reasonable purity. The most noticeable difference between the products of different manufacturers is the considerable variation in the amount of water of crystallisation, especially in the magnesium and 50% sodium salts. The majority are not adjusted to a basis of 50% of anhydrous salt. The 50% potash salt appears to be.—G. J. W. Ferrey, *Yearb. Pharm.*, 1926, 481.

Determination of Orthophosphates in Glycerophosphates. This can be effected by precipitation as ammonium phosphomolybdate under conditions calculated to secure complete precipitation.

Solution A—Molybdic anhydride 100 g., strong solution of ammonia 120 ml. water 300 ml. Dissolve by warming, add 380 ml. of water and cool.

Solution B—Concentrated nitric acid 300 ml., ammonium nitrate 20 g., water 900 ml. Add solution A to solution B with constant stirring, allow to stand 24 hours and filter through a fine paper immediately before use. Store in a cool place. Place about 1 to 5 g. of sample (sufficient to give about 0·15 g. of precipitate), accurately weighed, in a 150 ml. beaker and add 10 ml. of water. For the Na, Ca and Mn salts add nitric acid drop by drop to complete solution and render it acid to methyl orange; in the case of the ferric salt warm on a steam-bath to dissolve, stirring constantly. If solution is incomplete, filter. In a second beaker warm 100 ml. of the reagent to 55° on a steam-bath. Pour the warm reagent into the sample, stir, allow to stand 15 to 20 minutes, filter through a Gooch crucible dried at 120° to 130°, wash thoroughly with water acidified with 5 ml. of nitric acid per 100 ml., dry at 120° to 130° and weigh. Wt. of yellow precipitate $\times 0\cdot0376$ = wt. of orthophosphate as P_2O_5 .—R. M. Hitchens and M. S. McCauley, *J. Amer. pharm. Ass.*, 1936, 990.

Calcii Glycerophosphas (B.P.C.). $\text{CaC}_3\text{H}_5(\text{OH})_2\text{PO}_4\cdot 2\text{H}_2\text{O}=246\cdot2$. Loses not more than 15% of its weight at 130° and then

contains not less than 98% of the anhydrous salt, determined by ignition to pyrophosphate. Yields not more than 1% of residue (glycerin, etc.) insoluble in dehydrated alcohol; limits of alkalinity or acidity, citrate and glycerin and minimum of glycerophosphate are prescribed. The *N.F. VII* allows a loss of only 10% at 130° and is then of the same strength; assayed by precipitation of the calcium as oxalate in acetic acid solution and subsequent ignition to oxide. Glycérophosphate de Calcium, *Fr. Cx.* 1937, loses at 150° for 4 hours, not more than 15% moisture. The dried salt yields on incineration not less than 58% of pyrophosphate, contains by titration with N/10 sulphuric acid not less than 90% of the anhydrous substance, and by precipitation with magnesia mixture contains not less than 14.2% of phosphorus. Calcium glycerinophosphoricum, *P.G. VI*, contains not less than 84% of anhydrous calcium glycerophosphate, by titration with N/1 hydrochloric acid to methyl orange, addition of phenolphthalein and titration with N/1 potash; the two titrations should be equal. Calcium glycerinophosphoricum, *P. Helv. V*, contains not more than 16% of moisture, not less than 84% of $C_3H_7O_6PCa$, determined by dissolving in normal hydrochloric acid and titrating back with sodium hydroxide to methyl orange, and from 50.6 to 55.5% of ash on ignition.

Ferri Glycerophosphas (*B.P.C. Supp. IV*). Assayed for Fe content by the method of the *B.P.* for Ferri et Ammonii Citras, contains from 13 to 16% of Fe. Residue on ignition, not less than 42%. Ferri Glycerophosphas, *N.F. VII*, loses not more than 10% at 130° and the dried substance contains not less than 17% of Fe, equivalent to not less than 95% of $Fe_2(C_3H_7O_6P)_3$; in assaying, sodium bicarbonate is added to the acid mixture with potassium iodide.

Magnesii Glycerophosphas (*B.P.C.*).

$MgC_3H_5(OH)_2PO_4 \cdot 2H_2O = 230.4$. Loses not more than 16% at 130°, and then contains not less than 97% of $C_3H_7O_6PMg$, as estimated by weight of the ignited residue of pyrophosphate. Similar limit tests to those for the calcium salt are described.

Mangani Glycerophosphas (*B.P.C.*). $MnC_3H_5(OH)_2PO_4 = 225.0$. By calculation from the weight of the ignited residue the substance dried at 130° contains not less than 97%. Loss at 130° not more than 5%. Tests for limit of citrate, glycerin, alkalinity or acidity, and minimum of glycerophosphate are included. Mangani Glycerophosphas, *N.F. VII*, loses not more than 10% at 110°, and then contains not less than 98% of $MnC_3H_5(OH)_2PO_4$ when assayed by precipitation with dibasic ammonium phosphate in alkaline solution and ignition to $Mn_2P_2O_7$.

Assay. (*N.F. VII*.) Accurately weigh approximately 1.0 g., previously heated to constant weight, and dissolve in 1.5 ml. of nitric acid and 5 ml. of warm distilled water. Dilute to 125 ml., add 2.0 g. of dibasic ammonium phosphate, and heat to boiling; add a few drops of methyl red indicator and add strong ammonia solution drop by drop until alkaline, and then add 2.0 ml. excess. Allow to stand 2 hours at room temperature. Filter on to a Gooch

crucible and wash with a dilute solution of ammonia (1 in 100). Dry at 110° and ignite at a bright red heat. Each g. of $Mn_2P_2O_7$ is equivalent to 1.5855 g. of $MnC_4H_4(OH)_2PO_4$.

Potassii Glycerophosphas Liquidus (*B.P.C. Supp. IV*). Determined by the same methods as Sodii Glycerophosphas, it complies with the same limits for free alkali, free phosphate and glycerin and contains 48 to 52% *w/w* of $C_3H_7O_6PK_2 \cdot 3H_2O$. Sp. gr., 1.34 to 1.40.

Sodii Glycerophosphas (*B.P.C.*). $C_3H_7O_6PNa_2 \cdot 5\frac{1}{2}H_2O = 315.2$. Contains not less than 98% and not more than the equivalent of 102% of the pure substance with free alkali limit (as Na_2CO_3) of 0.5% and a free phosphate limit (as P_2O_5) of 0.5%. Assayed by the method of the *B.P.C.* by titration with normal hydrochloric acid using bromocresol green as indicator, less the sum of the readings for free alkali and free phosphate, these being obtained by titration with normal hydrochloric acid to thymol blue (free alkali) and subsequent addition of neutralised calcium chloride solution, boiling, and further titration of the cooled liquid with normal sodium hydroxide to thymol blue (free phosphate). The *N.F. VII* substance contains 68 to 74% of the anhydrous salt, as shown by titration with hydrochloric acid to methyl orange. Glycerophosphate de Sodium Cristallisé, *Fr. Cx.* 1937, contains 5 molecules of water of crystallisation; by titration with *N/10* sulphuric acid to helianthin it contains not less than 66% of the anhydrous salt and leaves 42% of residue on ignition.

Sodii Glycerophosphas Liquidus (*B.P.C.*). A solution of sp. gr. 1.28 to 1.32 containing 48 to 52% by weight of $C_3H_7O_6PNa_2 \cdot 5\frac{1}{2}H_2O$; free alkali (as Na_2CO_3) and free phosphate (as P_2O_5) limits, 0.5%. Assayed by the same process as for Sodii Glycerophosphas. Limit of free glycerin, determined by shaking with four volumes of dehydrated alcohol and one part of recently ignited calcium sulphate, filtering and evaporating the filtrate and alcoholic washings, and drying at 70° for one hour, 2%. Refractive index, 1.395 to 1.405.

ACIDUM HYDRIODICUM

(WITH METALLIC IODIDES)

Acidum Hydriodicum Dilutum (*B.P.C.*). Contains 9.8 to 10.2% *w/w* of HI, replacing the limits of the *B.P.* '14, approximately 9.7 to 10.2%, and a sulphate limit is included. Assayed by addition of excess silver nitrate solution and titration with ammonium thiocyanate. The *U.S.P. XII* acid contains from 9.5 to 10.5% *w/v* of HI and from 0.6 to 1.0% *w/v* of H_3PO_2 . Hypophosphorous acid is determined, after oxidation with hydrogen peroxide and volatilisation of the iodine, by precipitation with magnesia mixture and ignition to pyrophosphate. Tests for free iodine and limit of hydrochloric acid are included.

Syrupus Acidi Hydriodici (U.S.P. XII). Contains 1.3 to 1.5% *w/v* of HI.

Ammonii Iodidum (B.P.C.). $\text{NH}_4\text{I} = 145.0$. The salt dried at 100° contains 98%, 5% loss on drying at 100° being allowed. Assayed by the B.P. process for potassium iodide by titration with potassium iodate in presence of hydrochloric acid. The N.F. VII dries the salt for 6 hours at 110° , the limit of moisture and the standard then being the same, the use of 1% of ammonium hypophosphite as a stabilising agent is allowed. Assayed by digestion on a water-bath until the precipitate with standard silver nitrate solution is yellow, cooling and titrating with standard ammonium thiocyanate.

Calcii Iodidum (B.P.C.). $\text{CaI}_2 = 293.9$. Determined by iodide content it contains not less than 80% of CaI_2 .

Syrupus Ferri Iodidi (B.P.). Contains 4.75 to 5.25% *w/w* of FeI_2 . The preparation of the N.F. VII contains 6.5 to 7.5% *w/v* of FeI_2 , equivalent to approximately 5% *w/w* of FeI_2 .

Potassii Iodidum (B.P.). $\text{KI} = 166.0$. Loses not more than 1% at 110° and then contains 99% of KI when determined by titration with M/20 potassium iodate in the presence of not less than 40% of hydrochloric acid. The U.S.P. XII salt complies with the same standards and is assayed similarly, but the titration mixture is allowed finally to stand 5 minutes before adjusting the end-point. The Fr. Cx. 1937 requires a purity of 99% on the dried salt and alkalinity not in excess of 0.8% of KOH.

Assay. The following assay is recommended by the Sub-Committee on Inorganic Chemicals of the Committee on General Chemistry: Dissolve about 0.7 g., accurately weighed, in 50 ml. of water, add 15 ml. of hydrochloric acid and 6 ml. of solution of potassium cyanide, and titrate with M/20 potassium iodate until the dark-brown solution, which is formed, becomes pale yellow, then add 5 ml. of mucilage of starch and continue the titration slowly until the liquid becomes colourless. Each ml. of M/20 potassium iodate is equivalent to 0.0166 g. of KI. (*British Pharmacopœia Commission Report*, No. 14, September 1939.)

Alternative Assay Process. The following assay process based on the formation of iodine cyanide is preferable to that of the B.P. The potassium iodide solution, containing about 0.5 g. in 50 ml. of water, is strongly acidified with hydrochloric acid and treated with potassium cyanide solution. The mixture is then titrated with M/40 potassium iodate, using starch mucilage as indicator, until the last trace of blue or reddish violet colour has disappeared. Each millilitre of M/40 potassium iodate is equivalent to 0.0083 g. of KI.

This method can also be applied to solutions containing iodine and potassium iodide. 5 ml. of approximately N/10 iodine is diluted to about 100 ml. and treated with 50 ml. of 2N hydrochloric acid and 5 ml. of 10% potassium cyanide. The mixture is then titrated with M/40 potassium iodate until the colour disappears. Excess of potassium iodide is then added and the liberated iodine titrated with thiosulphate. If the two titrations are *a* and *b* ml. respectively, the potassium iodide is given by the expression

$$\frac{166.02 (5a - 2b)}{200} \text{ g. per litre,}$$

and the iodine is given by the expression

$$\frac{126.92 (2b - 3a)}{100} \text{ g. per litre.}$$

—A. D. Mitchell, Lecture to Institute of Chemistry, 19/10/1934.

Tabellæ Potassii Iodidi (N.F. VII). Tablets of 0.3 g. or more contain 94 to 106%, and tablets of less than 0.3 g. contain 92.5 to 107.5%, of the labelled amount of potassium iodide, including all tolerances. Assayed by titration with M/50 potassium iodate.

Sodii Iodidum (B.P.). $\text{NaI} = 149.9$. Loses not more than 5% at 110° and then contains 99% of the pure salt. It is assayed by titration with M/20 potassium iodate in the presence of hydrochloric acid. The U.S.P. XII allows 5% of water loss at 120° and the salt should then be of 99% purity, assayed by titration with

potassium iodate, the final adjustment of the end-point being made after allowing the titration mixture to stand for 5 minutes. The *Fr. Cx.* 1937 requires the dried salt to contain 98% of NaI and to possess alkalinity not more than 0.6% as NaOH by titration to phenolphthalein.

Strontii Iodidum (B.P.C.). $\text{SrI}_2 \cdot 6\text{H}_2\text{O} = 449.6$. Titrated with standard iodate solution, it contains 99% of the hydrated substance.

Zinci Iodidum (B.P.C.). $\text{ZnI}_2 = 319.2$. Contains not less than 98% of the pure salt, assayed for its iodide content. The *N.F. VII* salt is of the same strength after losing not more than 5% over sulphuric acid for 24 hours.

Calcii Iodobehenas (U.S.P. XII). Loses not more than 2% at 100°, and then contains not less than 23.5% of I; assayed by extraction with water of the residue obtained on ignition with potassium carbonate, addition of potassium permanganate to a pink coloration to the liquid on a water-bath, decolorising with alcohol, cooling and filtering, followed by titration, with sodium thiosulphate, of the iodine liberated in an aliquot part by potassium iodide and sulphuric acid.

Iodised Salt.

DETERMINATION OF IODINE. 100 g. of the salt is dissolved in water, the solution diluted to 500 ml. and filtered. To 200 ml. of the filtrate, made faintly acid to methyl orange, is added 1 ml. of bromine water. The solution is boiled until salt begins to separate, pumice being added to prevent bumping. Sufficient water is added to dissolve the precipitate, then 2 ml. of N/1 hydrochloric acid and 0.2 g. of potassium iodide, and the liberated iodine titrated with N/500 sodium thiosulphate. Unless the iodide is oxidised to iodate in practically neutral solution the reaction is incomplete in the presence of excess of sodium chloride.—R. L. Andrew and J. L. Mandeno, *Analyst*, 1935, 801.

Iodide is lost when the iodised salt is stored in cardboard containers owing to absorption by the cardboard; there is no loss to the atmosphere. The loss may amount to 50% during 6 months.—R. C. Andrews, *Analyst*, 1938, 180.

ACIDUM HYDROBROMICUM

(WITH METALLIC BROMIDES)

Acidum Hydrobromicum (B.P.C.). Contains 34 to 35% w/w of HBr and has sp. gr. of 1.303 to 1.314. 290 g. with 710 g. of water produces an acid of approximately the same strength as Acidum Hydrobromicum Dilutum, *B.P.* '32, containing 9.8 to 10.2% of HBr and with a sp. gr. of 1.072 to 1.075; a limit of chlorine equivalent to about 0.35% w/w of HCl is therefore included. Acidity estimated with standard sodium hydroxide to methyl orange.

Ammonii Bromidum (B.P.C.). $\text{NH}_4\text{Br} = 97.96$. Loses not more than 1% at 100° and then contains 99% of the pure salt. The *B.P.* '14 required only 98% purity with the same moisture allowance. Estimated by total halogen content, with chloride and bromate limits specified. The *N.F. VII* salt contains 99% of NH_4Br , after drying 24 hours over sulphuric acid. Bromure d'Ammonium, *Fr. Cx.* 1937, dried at 100°, contains, when assayed gravimetrically, not less than 98% of NH_4Br . Ammonium bromatum, *P.G. VI*, dried at 100°, contains at least 98.8% of NH_4Br .

Calcii Bromidum (*B.P.C. Supp. IV*). $\text{CaBr}_2 \cdot 2\text{H}_2\text{O} = 235.9$. Contains 95 to 102% of the pure salt, assayed as Potassii Bromidum. Calcii Bromidum, *N.F. VII*, contains 84 to 94% of the anhydrous salt, which is equivalent to approximately 99 to 111% of the hydrated compound; assayed for calcium content by precipitation with ammonium oxalate and titration of the precipitate in acid mixture with N/10 potassium permanganate. The *Fr. Cx.* 1937 substance, assayed by weight of silver bromide, contains not less than 80% CaBr_2 .

Potassii Bromidum (*B.P.*). $\text{KBr} = 119.0$. Loses not more than 1% at 110° and then contains 99% of the pure salt. Assayed by addition of excess silver nitrate and back titration with ammonium thiocyanate. It is official in the *U.S.P. XII*. Bromure de Potassium, *Fr. Cx.* 1937, by weight of silver bromide, yields 99% of KBr on the dried substance.

Sodii Bromidum (*B.P.*). $\text{NaBr} = 102.9$. Loss on drying at 110° not more than 5% and then contains 99% of the pure salt, assayed for bromide content. The *U.S.P. XII* requires the substance dried at 110° to contain 99% of NaBr ; moisture, not more than 1%. Bromure de Sodium, *Fr. Cx.* 1937, loses not more than 2% at 100° and then contains not less than 97% of pure salt.

Tabellæ Sodii Bromidi (*N.F. VII*). Contain 92.5 to 107.5% of the labelled amount of sodium bromide, including all tolerances. Comply with a limit test for chloride. Assayed by adding excess N/10 silver nitrate and nitric acid and titrating with N/10 ammonium thiocyanate.

Tabellæ Bromidum Trium (*N.F. VII*). Contain bromine equivalent to 70 to 81% of the labelled amounts of total bromides (equal proportions of ammonium, potassium and sodium bromides), and contain 30.8 to 35.8% of ammonium bromide, including all tolerances in both cases. Assayed for total bromine by dissolving in water, adding excess N/10 silver nitrate and titrating with N/10 ammonium thiocyanate in the presence of nitric acid. Assayed for ammonium bromide by dissolving in water, adding sodium hydroxide solution, distilling into N/2 sulphuric acid and titrating the excess acid with N/10 sodium hydroxide using methyl red indicator.

Strontii Bromidum (*B.P.C.*). $\text{SrBr}_2 \cdot 6\text{H}_2\text{O} = 355.6$. Assayed by the same method as Potassii Bromidum. It should contain not less than 97% of the pure substance. The *N.F. VII* salt should contain not less than 98% of the hexahydrate; loss at 200°, not more than 32%. Bromure de Strontium, *Fr. Cx.* 1937, contains not less than 69.5% of the anhydrous salt.

Zinci Bromidum (*B.P.C. Supp. IV*). $\text{ZnBr}_2 = 225.2$. Contains not less than 95% of ZnBr_2 . Assayed by the *B.P.* process for Zinci Sulphas as follows:—Dissolve about 0.3 g. in 120 ml. of water, acidify with a drop of dilute sulphuric acid, add 25 ml. of mercuric ammonium thiocyanate solution, allow to stand for 5 minutes, stir well and stand for a further hour; filter on a small suction filter and wash with five quantities of 10 ml. of mercuric ammonium thiocyanate solution, diluted with water (1 : 50). To the precipitate and filter in a stoppered bottle add 5 ml. of water and 40 ml. of hydrochloric acid and titrate immediately with M/5 potassium iodate, using 5 ml. of chloroform as indicator.

ACIDUM HYDROCHLORICUM

(with METALLIC CHLORIDES)

Acidum Hydrochloricum (B.P.). Contains from 31 to 33% by weight of HCl; sp. gr., 1.156 to 1.168. Acidum Hydrochloricum Dilutum contains from 9.5 to 10.5% w/w of HCl and has a sp. gr. of 1.045 to 1.052. The stronger acid of the *U.S.P. XII*, having a sp. gr. at 25° of about 1.18, should contain 35 to 38% w/w of HCl. The dilute acid, sp. gr. 1.046 at 25°, contains 9.5 to 10.5% w/v of HCl. *Acide Chlorhydrique Officinal, Fr. Cx.* 1937, contains not less than 35.5% of HCl. Acidum hydrochloricum fortius and Acidum hydrochloricum dilutum, *P. Helv. V*, should contain respectively 24.9 to 25.1% and 9.9 to 10.1% w/w of HCl. Acidum hydrochloricum, *P.G. VI*, contains 24.8 to 25.2% of HCl and Acidum hydrochloricum dilutum 12.4 to 12.6%.

The British Standards Institution has published British Standard Density-Composition Tables for Aqueous Solutions of Hydrochloric Acid (*B.S.S. No. 976—1941*) giving the concentration in g. of HCl per 100 g. and in g. of HCl per 1000 ml. corresponding to densities of 1 to 1.161 for each 5° over the range of 0° to 40°. The tables are designed for use in conjunction with British Standard Density Hydrometers.

Ammonii Chloridum (B.P.). $\text{NH}_4\text{Cl} = 53.5$. Loses not more than 1% when dried in a vacuum desiccator over sulphuric acid for 24 hours and should then contain 99.5% of NH_4Cl . Residue on volatilisation not more than 0.1%. Assayed on the chloride content by addition of excess standard silver nitrate to a solution acidified with nitric acid and adjustment to volume, followed by titration of an aliquot part with ammonium thiocyanate using ferric ammonium sulphate as indicator. The salt is official in the *U.S.P. XII* and is of the same strength. Chlorure d'Ammonium, *Fr. Cx.* 1937, determined gravimetrically, contains not less than 99% of NH_4Cl .

Improved Method of Assay. Dissolve about 9.14 g., accurately weighed, in water, dilute to 50 ml., add 8 to 10 drops of a 0.1% aqueous solution of sodium fluorescein, and titrate with N/10 silver nitrate solution, shaking after each addition. The end-point is the formation of a pronounced pink tint, and is not affected by adsorption as in the Volhard Method. The method is rapid and gives more accurate results than the official method.—T. L. Bowyer, *Chem. & Drugg.*, ii/1939, 343.

Tabellæ Ammonii Chloridi (N.F. VII). Contain 94 to 106% of the labelled amount of ammonium chloride for tablets of 0.3 g. or more and 92.5 to 107.5% for tablets of less than 0.3 g., including all tolerances. Assayed by dissolving a weighed quantity of powdered tablets in water, adding excess N/10 silver nitrate, filtering and titrating with N/10 ammonium thiocyanate.

Calcii Chloridum (B.P.). $\text{CaCl}_2 = 111.0$. The salt should be of 98% purity after drying at 200°, when the loss should be not more than 10%. Assayed by titration with standard silver nitrate using potassium chromate as indicator.

Calcii Chloridum Hydratum (B.P. Add. I). $\text{CaCl}_2 \cdot 6\text{H}_2\text{O} = 219.1$. Contains 98% to the equivalent of 102% of the hydrated salt by titration with silver nitrate. Calcii Chloridum, *U.S.P. XII*, the hydrated salt with 2 molecules of water, contains 75 to 81%

of CaCl_2 . Determined by precipitation with excess standard oxalic acid in ammoniacal solution followed by titration of the acidified filtrate with potassium permanganate.

British Standard Density-Composition Tables for Aqueous Solutions of Sodium Chloride and of Calcium Chloride (*B.S.S.* No. 823—1938) give the concentration in g. of CaCl_2 per 100 g. and in g. of CaCl_2 per 1000 ml. corresponding to densities of 1.00 to 1.39 for each 5° over the range of 10° to 40° . The tables are designed for use in conjunction with British Standard Density Hydrometers.

Magnesi Chloridum (B.P.C.). $\text{MgCl}_2 \cdot 6\text{H}_2\text{O} = 203.3$. 2 g. yields a clear solution in 10 ml. of alcohol (85%); CaSO_4 limit, 4%, determined by dissolving in 25 parts of 20% sulphuric acid, adding 50 parts of 95% alcohol and allowing to stand overnight.

Potassii Chloridum (B.P.C.). $\text{KCl} = 74.55$. Titrated by the method for Sodii Chloridum, the substance dried at 130° contains 99.5% of KCl . Loses not more than 1.0% at 130° . Potassii Chloridum, *U.S.P. XII*, loses not more than 1% at 110° , and then contains 99% of KCl .

Detection of Manganese in Presence of Chlorides. Dissolve 25 g. of sample in 100 ml. of hot water, add 5 ml. of hydrochloric acid and boil. If iron is absent, add 0.1 g. of ferric ammonium sulphate. Then make the solution just alkaline with 20 per cent. sodium hydroxide solution and continue boiling to coagulate the ferric hydroxide. Collect the precipitate and dissolve it by adding two 10-ml. portions of 10% sulphuric acid, collecting the solution in a 20-ml. conical flask. Add 2 g. of phosphoric acid and 0.5 g. of potassium periodate and boil for twenty minutes. Compare the resulting coloured solution with standard permanganate solutions.—R. Gilbert, *Analyst*, 1941, 450.

Tabellæ Potassii Chloridi (U.S.P. XII). Contain 95 to 105% of the labelled amount of KCl , including all tolerances. Comply with tests for calcium, magnesium and sodium. Assayed by dissolving in water, adding excess N/10 silver nitrate and back titration with N/10 ammonium thiocyanate.

Sodii Chloridum (B.P.). $\text{NaCl} = 58.45$. Loses not more than 1.0% at 130° and then contains 99.5% of the pure salt. A test for the absence of iodides and bromides, by the addition of chloroform and chlorine solution to the alcohol-soluble matter dissolved in water, is included. The *U.S.P. XII* salt contains 99.5% of NaCl after drying at 110° . 5 g. in 50 ml. of water requires not more than 0.1 ml. N/50 sodium hydroxide to produce a blue colour, or not more than 0.2 ml. of N/50 hydrochloric acid to produce a yellow colour, with bromothymol blue. The *Fr. Cx.* 1937 requires a purity of 99% on the dried substance.

The Food and Drug Administration of the U.S. Dept. of Agriculture defined table salt, dairy salt, as fine-grained crystalline salt containing, on a water-free basis, not more than 1.4% of calcium sulphate, not more than 0.5% of calcium and magnesium chlorides, nor more than 0.1% of matter insoluble in water. Pending further announcement, no exception is taken to content of anhydrous calcium sulphate (anhydride) in excess of 0.1% provided the total calcium sulphate content does not exceed 1.4%.—*S.R.A., F.D. No. 2, Rev. 5, Nov. 1936.*

British Standard Density-Composition Tables for Aqueous Solutions of Sodium Chloride and of Calcium Chloride (*B.S.S.* No. 823—1938), designed for use in conjunction with British Standard Hydrometers, give the concentration in g. of NaCl per 100 g. and in g. of NaCl per 1000 ml. corresponding to densities of 1.00 to 1.200, for each 5° over the range of 0° to 40° .

Liquor Sodæ Chlorinatæ Chirurgicæ (B.P.). Contains 0.5 to 0.55% w/v of available chlorine, determined by interaction with potassium iodide and titration with N/10 sodium thiosulphate.

Chlorine in Industry.

This substance is encountered in industry in bleach and alkali works and is also used largely from cylinders, which are apt to leak or to stick, and it is in attending to these two troubles that there is the greatest risk of an accident. There is no risk of delayed action and on account of its lower solubility it is about 20 times as toxic as hydrochloric acid, which is much more rapidly absorbed into the system. It is a greenish-yellow gas with a distinctive odour, which can be detected in a concentration of about 3 to 5 parts per million. A concentration of 1 in 1000 is rapidly fatal and the greatest possible concentration for prolonged exposure would appear to vary between 1 and 10 parts per million. Workers appear to become acclimatised to the vapours of chlorine and not to develop hypersensitivity.

Treatment. If there is any risk of exposure to a dangerous concentration the patient should be rested for 24 hours and kept warm. As a rule drugs are not required but if pulmonary complications set in these should be treated accordingly. In mild cases ordinary expectorants may be useful when acute symptoms have abated.

Detection and Determination. Chlorine may be detected by the development of a blue colour with aniline acetate or a yellow colour with *o*-tolidine and also by the liberation of iodine from starch-potassium iodide solution of paper. For its determination in the atmosphere the most satisfactory method is to draw the suspected air through a solution containing 0.1% of *o*-tolidine in a solution containing 10% of concentrated hydrochloric acid and comparing the yellow colour developed with a standard dichromate solution or a solution of the reagent to which a known amount of chlorine has been added.—(*Toxic Gases in Industry*, No. 10, D.S.I.R.)

Zinci Chloridum (B.P.). $\text{ZnCl}_2 = 136.3$. Assayed by precipitation of the solution acidified with sulphuric acid with mercuric ammonium thiocyanate, and titration in hydrochloric acid of the washed precipitate with standard potassium iodate, using chloroform as indicator, it contains zinc equivalent to 95% of ZnCl_2 . The U.S.P. XII salt is of the same strength, determined by precipitation as carbonate and ignition to oxide.

Perchloric Acid, HClO_4 . Available commercially containing 60% *w/w*, with sp. gr. 1.54. A powerful acid and oxidising agent. As a reagent for potassium, qualitatively and quantitatively, it has largely replaced the chloroplatinate method.

Mercurimetric Determination of Halides. The method is based on the precipitation of the mercurous halide when a solution of mercurous nitrate is added to that of the halide. Approximately N/10 mercurous nitrate is prepared by dissolving about 30 g. of the salt in a litre of N/5 nitric acid. The solution when kept in contact with a globule of mercury is stable for several months. The solution is standardised against N/10 sodium chloride, using ferric thiocyanate as indicator. 1 ml. of N/20 ammonium thiocyanate and 2 ml. of a concentrated solution of ferric nitrate are added to 25 ml. of N/10 sodium chloride and the solution titrated with the mercurous nitrate solution until disappearance of the red colour. To correct for the volume of mercurous nitrate

solution required by the indicator, a blank titration is made with an equal volume of the indicator and the volume obtained is subtracted from that obtained in the standardisation. The determination of chlorides and bromides is carried out by the above method. For the determination of iodides, 25 ml. of an approximately N/10 solution of the iodide is added to 50 ml. of N/10 mercurous nitrate, the solution is filtered and the precipitate washed several times with water acidified with a little dilute nitric acid. The excess of mercurous nitrate in the filtrate is determined by oxidation to mercuric nitrate and titration with ammonium thiocyanate. The filtrate is treated with 2 ml. of dilute nitric acid and sufficient saturated solution of potassium permanganate to produce a pink coloration, the excess of permanganate is removed with a little ferrous sulphate solution and then the resulting solution titrated with N/10 ammonium thiocyanate.—M. Chitchigol, per *Quart. J. Pharm.*, 1937, 89.

Detection of Halides in Presence of Cyanides. Excess of 5% mercurous nitrate solution is added to the precipitate obtained with silver nitrate and nitric acid; silver cyanide becomes black in colour and then dissolves. Any silver halides present are unacted upon and can be identified.—G. A. D. Haslewood, *J. chem. Soc.*, 1936, 1049.

Detection of Halides in Presence of Thiocyanates. To 10 ml. of sample add 1 ml. of carbon tetrachloride and 0.5 g. of potassium persulphate. If iodide is present the iodine liberated colours the carbon tetrachloride violet. The aqueous layer is separated and neutralised with 2N sodium carbonate and an excess of 15 ml. added, and the mixture evaporated to dryness. The residue is dissolved in 10 ml. of water and the iodine completely removed by warming with dilute nitric acid and potassium persulphate and extracting with carbon tetrachloride. To the cold iodine-free solution add 4 ml. of nitric acid and 1 ml. of carbon tetrachloride. A yellow coloration of the solvent which persists after allowing to stand for one minute with occasional stirring indicates presence of bromine. The aqueous layer is again separated and boiled for 1 minute with excess of potassium permanganate to remove bromide, sulphate is removed with barium nitrate and the filtrate is tested for chloride with silver nitrate.—G. B. and L. K. Heisig, *Industr. Engng Chem. (anal. Edn.)*, 1935, 249.

Determination of Cyanide, Chloride and Iodide Mixtures. Cyanide is determined by a direct titration with silver nitrate without any indicator, the end-point being the appearance of a permanent opalescence which is best seen by placing a black surface under the titration vessel. End-points are obtained without difficulty at so low a concentration as N/50. A second portion of the sample is boiled for 15 minutes with a slight excess of potassium bitartrate to eliminate the cyanide and the iodide is determined by titration of the cooled solution with silver nitrate, using rose bengal as adsorption indicator. The liquid is decanted through a filter, the precipitate washed with very dilute nitric acid and the chloride determined by titrating the filtrate with silver nitrate using phenosafranine as indicator. Tartrazine can be used instead of phenosafranine; both indicators give satisfactory results for the titration in nitric acid solution up to an acid concentration of about normal, but phenosafranine is preferable for work at extreme (N/100) dilution. Mixtures of cyanide and chloride or bromide may be determined after direct titration of the cyanide as above by adding nitric acid and excess of silver nitrate, filtering off the precipitate, and back-titrating the excess of silver nitrate with potassium bromide using either phenosafranine or tartrazine as indicator. The method is also applicable to the determination of thallous-thallic halides for which Volhard's method is unsatisfactory. The sample is reduced with a 2% zinc amalgam and the reduced solution is run from a burette into a known volume of silver nitrate solution using tartrazine as indicator.—A. J. Berry, *Analyst*, 1936, 315.

ACIDUM HYDROCYANICUM

Acidum Hydrocyanicum Dilutum (B.P.). Contains 1.9 to 2.1% w/w of HCN. **Acidum Hydrocyanicum Fortius (B.P.C. '34)** contains 3.8 to 4.2% of HCN, and the dilute acid of the International Agreement, 2%. Determined by titration with silver nitrate in ammoniacal solution using potassium iodide as

indicator. Acidum Hydrocyanicum Dilutum, *N.F. VII*, contains 1.9 to 2.1% *w/w* of HCN, and not more than 0.1% of HCl. Determined by titration with silver nitrate in alkaline solution, using potassium iodide as indicator, and titration of total halide by addition of excess silver nitrate, filtration and titration with ammonium thiocyanate, the difference between the two titrations being calculated as HCl.

Determination of hydrocyanic acid.—C. E. Corfield and C. J. Eastland, *Yearb. Pharm.*, 1921, 391.

Phthalin Method of Estimation. The most suitable method for the estimation of traces of hydrocyanic acid in cyanogenetic plants is that depending on the oxidation of a phthalin to a coloured phthalein. Using *o*-cresolphthalin, 1 part of hydrocyanic acid in 100 million can be detected with certainty. Reagents required include:—*Copper Reagent*.—150 mg. of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 100 ml. of water; *Sodium Sulphite Reagent*.—5 g. of anhydrous sodium sulphite and 0.224 g. of triethanolamine hydrochloride in 100 ml. of water; *Cresol Red Reagent*.—50 mg. of cresol red in 125 ml. of alcohol made up to 250 ml. with water. Five ml. of this solution are treated with 2 ml. of $\text{N}/2$ sodium hydroxide solution and 2 ml. of the copper sulphate and triethanolamine hydrochloride reagent and made up to 200 ml.; *Copper Sulphate and Triethanolamine Hydrochloride Reagent*.—300 mg. of copper sulphate and 896 mg. of triethanolamine hydrochloride in 100 ml. of water. *Method*.—The solution, containing 0.01 to 0.1 mg. of hydrocyanic acid, is treated in a graduated 50 ml. flask with $\text{N}/2$ sodium hydroxide so that 1.5 ml. of $\text{N}/2$ NaOH is finally present; the volume is adjusted to 45 ml. and the flask cooled. The temperature is taken, 1 ml. of cresolphthalin reagent (40 mg. of *o*-cresolphthalin in 10 ml. of alcohol made up to 20 ml. with water) added, and the whole mixed. Then 1 ml. of copper reagent is quickly added, the flask stoppered and shaken; after exactly 5 minutes (measured by stopwatch) 2 ml. of the sodium sulphite reagent are rapidly added to stop the reaction, the contents of the flask mixed, made up to 50 ml. and transferred to the colorimeter, where the reading is taken at the end of 5 minutes. Comparisons are made with the alkaline mixture of cresol red, copper sulphate and triethanolamine hydrochloride, and a calibration curve of concentration against colorimeter reading is plotted. Temperature has a noticeable effect on colour development and the estimation should be made within 1° of the temperature to which the curve relates. Temperature control is necessary only during colour development and is not required after addition of the reducing agent.—R. I. Nicholson, *Analyst*, 1941, 189.

Determination in *Prunus Serotina*. From 15 to 25 g. of the powdered bark, accurately weighed, are allowed to stand with 200 ml. of water at ordinary temperature in a closed round-bottomed flask of 1 litre capacity and fitted with a side-arm for 24 hours. It is then steam-distilled into 1% sodium hydroxide solution for 1 hour. The distillate is acidified with hydrochloric acid, and, after the addition of excess of sodium bicarbonate, titrated with $\text{N}/20$ iodine.—Fordos and Gellis, per *Aust. J. Pharm.*, 1940, 570.

Delicate Test for Hydrocyanic Acid. A few drops of phenolphthalin solution made alkaline with sodium hydroxide added to liquid to be tested. If red colour be produced on adding cupric sulphate solution 1 in 2000 (due to oxidation into phenolphthalein) hydrocyanic acid is proved to be present. Phenolphthalin is made by reducing phenolphthalein with zinc in alkaline solution.—*Pharm. J.*, 1/1905, 721.

Place 1 drop of 1:5 ammonia dilution on a microscopic slide and invert over tube containing the solution to be tested, together with a few drops of sulphuric acid. After a few minutes remove slide and place under microscope: on the addition of 1 drop of alloxan solution (made by boiling 0.1 g. of uric acid with 0.2 ml. of nitric acid and 0.2 ml. of water and diluting to 5 ml.) crystals of oxaluramide begin to form in a few minutes if the test solution contains a cyanide. Substitution of pyridine for ammonia renders test far more delicate so that a few drops of a solution containing 0.01 g. HCN per litre gives positive results.—per *Yearb. Pharm.*, 1927, 168.

Detection of Inhaled Hydrocyanic Acid. 250 ml. of blood from the lungs is steam-distilled until 15 ml. of distillate has been collected. The

following tests are made on separate 5 ml. quantities of distillate. *Prussian Blue Test.* Make alkaline with 2 to 3 drops of 5% sodium hydroxide solution and add 2 drops of 5% ferrous sulphate solution and 1 drop of 3% ferric chloride solution. Allow to stand for 2 minutes, heat gently and add just sufficient dilute hydrochloric acid to clear. In the presence of traces of cyanide a blue precipitate may settle out only after standing overnight. *Sulphocyanide Test.* Heat with 1 drop of ammonium sulphide solution for 15 minutes on a water-bath. Add dilute nitric acid until the yellow colour is just destroyed and then ferric alum solution drop by drop.—G. D. Elsdon and J. R. Stubbs, *Analyst*, 1937, 540.

Sometimes both the above tests fail and the following may be tried. Collect the distillate from blood or lung tissue in a conical flask, fix sulphur compounds with a few drops of lead acetate solution and make faintly acid with sulphuric acid. Place a drop of approximately N/10 silver nitrate on a microscope slide, invert over the mouth of the flask, and stand in a warm place for at least 30 minutes. Microscopic examination may reveal characteristic crystals of silver cyanide.—G. Roche Lynch, *ibid.*

As a bactericide (fumigant in ship disinfection) hydrocyanic acid is too weak to affect pathogenic germs. It has no measurable carbolic acid co-efficient. *Less than 0.02.*—W. C. Reynolds, *Lancet*, ii/1922, 834. Its danger.—*Pharm. J.*, i/1923, 300. It destroys rats, fleas, etc. It has no ill effects on dry grain, but moist food, e.g., butter, milk, etc., is liable to absorb the gas.

Insecticides. The specification of the Association of British Insecticide Manufacturers (Bulletin No. 82, Ministry of Agriculture and Fisheries) requires *Potassium Cyanide* to contain not less than 93% potassium cyanide; *Sodium Cyanide* to contain not less than 97% sodium cyanide; *Calcium Cyanide* to contain not less than 40% calcium cyanide. The strength of sodium cyanide is frequently expressed in terms of potassium cyanide; thus 97 to 98% sodium cyanide may be referred to as 129 to 130% cyanide.

Hydrogen Cyanide in Industry.

Hydrogen cyanide is extensively used in the fumigation of ships, flour mills, orchards, etc., and even rabbit burrows. It is also largely employed in gold extraction and in smaller amounts in other industries. In the last war it was tried as a war gas with indifferent success and it is doubtful whether its use would be repeated. For this purpose it was classed as a paralyzant. Hydrogen cyanide is sometimes formed in small quantities through the incomplete combustion of explosives. It is a colourless gas with a faint odour of bitter almonds, but can be noticed by some people by the dry taste at the back of the mouth. High concentrations are extremely rapid in their action, causing giddiness, headache and unconsciousness with convulsions. Its extreme rapidity of action is due to paralysis of the respiratory system, which prevents the use of oxygen by the tissues. One part in 500 is immediately fatal, whilst 1 part in 10,000 for an hour is very dangerous, and 1 part in 40,000 to 50,000 may cause symptoms after several hours' exposure. Milder symptoms of poisoning are irritation of the throat, headache, suffocation, nausea and giddiness.

Treatment. The patient should be removed immediately to fresh air and kept warm. If necessary, artificial respiration should be employed, using air mixed with 5 to 7% of carbon dioxide. Only partial success has so far attended the internal use of chemicals for neutralisation purposes.

Detection and Determination. Two methods are recommended. The benzidine-copper acetate method consists in soaking papers with a solution obtained by mixing 25 ml. of a freshly prepared solution of benzidine acetate (containing about 1%

of benzidine acetate) and 2 ml. of 3% cupric acetate solution. The papers are drained and nearly dried and air is drawn through them by means of a pump or aspirator, when a greyish-blue to a deep blue appears in the presence of hydrogen cyanide vapours. The depth and shade of colour may be compared with those of a standard chart and the amount of gas recorded. The Congo red-silver nitrate method depends on the use of freshly prepared papers, obtained by soaking in 0.05% Congo red solution, drying, subsequently soaking in 5% silver nitrate solution, and drying rapidly. The colour developed varies from a greyish-purple to a greyish-blue and by comparison with a standard chart the amount of gas in the atmosphere under examination may be determined.—(*Toxic Gases in Industry*, No. 2, D.S.I.R.)

Alkyl thiocyanates are of increasing use as insecticides, the following being found in commercial preparations. β -Butoxy-3-thiocyanodiethyl ether (Lethane 384), lauryl thiocyanate, 3-thiocyanopropylphenyl ether, trimethylenedithiocyanate, α -naphthyl isothiocyanate (Kessocide 95) and *m*-phenylene di-isothiocyanate. The first four appear to have a more paralysing action on cockroaches than the last two. Methods of analysis of preparations of normal aliphatic and aromatic thiocyanates are detailed based on a colorimetric procedure in terms of cyanogen. In the case of isothiocyanates, it is suggested that the most satisfactory method available for the quantitative determination is the utilisation of the same hydrolysis reaction used in the determination of normal thiocyanates and the subsequent determination of the primary amine.—W. E. Kemp, *Analyst*, 1939, 648.

Calcium Cyanide. Direct injection of rat harborages with calcium cyanide dust is simplicity itself. The dust is taken from a tightly covered can, in which it is marketed, and is poured into the container of a foot-pump type of duster, this container then being screwed into place. The rubber hose extending from the duster is inserted into small openings into harborages by a fumigator while another operates the pump. If the enclosed spaces to be treated are few or of limited extent, the whole operation might be carried on, even in the hold of a ship, without the wearing of gas masks by the fumigators. If, however, many harborages are to be treated or they are extensive, gas-mask protection is necessary. The material used, calcium cyanide, is at present manufactured in the United States under the trade-name Cyanogas. It is also manufactured in Germany, under the trade-name Calcyanide. Cyanogas is obtainable in flakes, granules, or as a dust. Calcyanide is at present obtainable only as a fine dust, although it is available abroad in the form of briquets, which are broken into a dust for use. Cyanogas is blue in colour, while Calcyanide is a light brown. Cyanogas contains from 40 to 50% of calcium cyanide, while Calcyanide contains from 80 to 85%. This is important to remember, since in interchanging these materials the Calcyanide must be used in approximately half the amounts of Cyanogas. For use in the foot-pump duster, the form of Cyanogas to be specified is the "A" dust.—*Publ. Hlth Rep., Wash.*, 1936, 139.

Aqua Laurocerasi (B.P.C.). Adjusted to contain 0.09 to 0.11% *w/v* of HCN and assayed by the method for Acidum Hydrocyanicum Dilutum. Aqua Laurocerasi I.A. and Aqua Amygdalæ Amaræ I.A. each contain 0.1% of HCN. The water of the *Fr. Cx.* 1937 should contain not more than 0.025% of free HCN and not less than 0.3% of benzoic aldehyde determined by weight of the separated phenylhydrazone. For medicinal use it contains 0.1% HCN, free and combined.

The bright green young *Prunus Laurocerasus* leaves were found to yield from two to four times the amount of hydrocyanic acid given by the older and more leathery brown leaves of cherry laurel. Adequate manuring caused increase in the amount of hydrocyanic acid contained.—D. H. Wester, *Pharm. J.*, 1/1904, 643.

ACIDUM HYPOPHOSPHOROSUM

Acidum Hypophosphorosum (B.P.C.). Contains 30 to 32% of H_3PO_2 . 323 g. with 677 g. of water yields an acid of approximately the same strength as Acidum Hypophosphorosum Dilutum, B.P., 9.8 to 10.2% w/w of H_3PO_2 . The U.S.P. XII acid is of the same strength as the B.P.C. acid.

Ammonii Hypophosphis (N.F. VII). $\text{NH}_4\text{H}_2\text{PO}_2 = 83.07$. Loses, over sulphuric acid for 24 hours, not more than 3% and then contains not less than 97.5% of $\text{NH}_4\text{H}_2\text{PO}_2$. Assayed by treatment with excess standard bromine solution in presence of sulphuric acid during 3 hours, addition of potassium iodide, and back titration with standard sodium thiosulphate; 1 ml. of N/10 bromine = 0.002076 g. of $\text{NH}_4\text{H}_2\text{PO}_2$.

Assay of Hypophosphites. About 0.7 g., accurately weighed, is dissolved in sufficient water to give 500 ml. To 50 ml. of the solution in a 250 ml. stoppered flask add 50 ml. of N/10 bromine and 20 ml. of dilute sulphuric acid; stopper the flask, shake well and allow to stand for 2 hours. Add 10 ml. of 20% w/v potassium iodide solution, shake, and titrate with N/10 sodium thiosulphate. Carry out a blank determination. 4 atoms of bromine are equivalent to 1 molecule of H_3PO_2 . This process is applicable to ammonium, potassium, calcium and manganese hypophosphites. For iron hypophosphite it must be modified as follows:—To about 0.15 g., accurately weighed, in a 200 ml. graduated flask, add 100 ml. of N/10 bromine and 20 ml. of dilute sulphuric acid; stopper the flask and shake well. Allow to stand, shaking occasionally until solution is complete. Add 30 ml. of 10% w/v sodium hydroxide solution, cool, and make up to volume. Filter, and to 100 ml. of filtrate add 10 ml. of 20% w/v potassium iodide solution and excess of dilute sulphuric acid, and titrate with N/10 sodium thiosulphate.—G. L. Jenkins and C. F. Bruening, *J. Amer. pharm. Ass.*, 1936, 19.

Tentative methods for the determination of hypophosphites in syrups are described in *Methods of Analysis (A.O.A.C., 1940, 617)*.

Calcii Hypophosphis (B.P.C. Supp. V). $\text{Ca}(\text{H}_2\text{PO}_2)_2 = 170.2$. Assayed by digestion in the dark for 12 hours with sulphuric acid and standard iodine solution and back titration with thiosulphate, a blank determination being made, it contains 98% of the pure substance; phosphate insoluble in water, not more than 0.5%. The N.F. VII substance is assayed similarly to the ammonium salt; 1 ml. of N/10 bromine = 0.002127 g. of $\text{Ca}(\text{H}_2\text{PO}_2)_2$; loss over sulphuric acid, not more than 3% and then contains 98% of the pure substance.

Ferri Hypophosphis (B.P.C. Supp. IV). $\text{Fe}(\text{H}_2\text{PO}_2)_3 = 250.9$. Contains from 97% to the equivalent of 101% of $\text{Fe}(\text{H}_2\text{PO}_2)_3$, after drying at 100°, when it loses not more than 5%. Assayed by the B.P.C. '34 method by digestion with potassium iodide and N/10 iodine in presence of hydrochloric acid in the dark for 4 hours, followed by back titration with N/10 sodium thiosulphate, by which the iodine liberated by the ferric radicle less that used in oxidising the hypophosphorous acid is titrated, a blank titration being made. Ferri Hypophosphis, N.F. VII, is estimated for Fe content only by evaporation with nitro-hydrochloric acid, interaction with potassium iodide in acid solution and titration of the liberated iodine, an Fe content on the dried substance of not less than 21.8% being indicated, equivalent to 98% of $\text{Fe}(\text{H}_2\text{PO}_2)_3$; loss over sulphuric acid, not more than 3%.

Mangani Hypophosphis (*B.P.C.*). $\text{Mn}(\text{H}_2\text{PO}_2)_2 \cdot \text{H}_2\text{O} = 203.0$. Estimated by hypophosphite content a purity of not less than 97% is required. As indicated by treatment with N/10 bromine solution and dilute sulphuric acid during 3 hours, and titration, with sodium thiosulphate, of the iodine liberated from potassium iodide, the *N.F. VII* salt dried over sulphuric acid for 2 hours is of the same purity.

Potassii Hypophosphis (*B.P.C.*). $\text{KH}_2\text{PO}_2 = 104.1$. Assayed by the *B.P.C.* method for Calcii Hypophosphis it contains 98% of KH_2PO_2 , after drying over sulphuric acid. Loses not more than 2% on drying over sulphuric acid. Insoluble phosphate limit, 0.5%. Potassii Hypophosphis, *N.F. VII*, assayed by the bromine absorption method, contains 98% of the pure substance after losing not more than 5% over sulphuric acid. 1 ml. of N/10 bromine = 0.002604 g. of KH_2PO_2 .

Sodii Hypophosphis (*B.P.C.*). $\text{NaH}_2\text{PO}_2 = 88.03$. By the assay process for Calcii Hypophosphis it contains 97% of the pure substance, calculated to the dry substance, a loss of 2% being allowed at 110°. Sodii Hypophosphis, *N.F. VII*, is the hydrated salt, $\text{NaH}_2\text{PO}_2 \cdot \text{H}_2\text{O}$, of which it should contain 98%, after drying for 2 hours over sulphuric acid; this loss should be not more than 3%; assayed by the bromine absorption method used for the ammonium salt of the *N.F. VII*. Hypophosphite de Sodium, *Fr. Cx.* 1937, by weight of the precipitate produced with mercuric chloride contains not less than 95% of the anhydrous substance.

ACIDUM LACTICUM

Acidum Lacticum (*B.P.*). Contains the equivalent of not less than 87.5% *w/w* of $\text{C}_3\text{H}_5\text{O}_3$; it is assayed to include both lactic acid and lactide, by boiling for five minutes with excess of normal sodium hydroxide and back titration with sulphuric acid to phenolphthalein, a blank titration being made. Sp. gr. about 1.21. A test for limit of various reducing sugars is included as shown by the formation of not more than a slight red precipitate on boiling the neutralised acid with Fehling's solution. The acid of the *B.P.* '14 contained 75% of hydrogen lactate with 10% of lactide. Lactic acid, *U.S.P. XII*, contains the equivalent of 85 to 90%; also assayed with excess of sodium hydroxide but with boiling for 20 minutes. Should give no reactions for sarcolactic acid with copper sulphate solution, or for citric, oxalic, phosphoric or tartaric acids with calcium hydroxide solution. The *P.G. VI* requires (with sp. gr. 1.206 to 1.216) total acid 90%, of which about 72% is free acid, reckoned as lactic acid. Assay process: 5 g. of lactic acid is diluted with water to 100 ml. 40 ml. of this mixture is neutralised with N/1 KOH in presence of phenolphthalein, using approx. 16 ml. of test solution (=72% of lactic acid). Further 5 ml. of N/1 KOH is added and the mixture warmed

5 minutes on the water-bath until the pink colour of phenolphthalein has disappeared. Then 2 ml. of N/1 HCl is added with further 2 minutes warming. The excess acid is back titrated. The total N/1 KOH used less the total N/1 HCl must be approx. 20 ml., i.e., approx. 90% total acid. *Acide Lactique Officiel, Fr. Cx.* 1937, assayed similarly, contains not less than 60% of lactic acid and not more than 30% of lactic anhydride. *Acidum lacticum, P. Helv. V.*, contains 88 to 92% w/w of total lactic acid. *Acidum Lacticum Dilutum, B.P.C.*, contains about 15% by volume of lactic acid and has a sp. gr. of about 1.04.

The absence from the *B.P.* of any limit for lactide is a disadvantage if the acid is used for the preparation of *Calcii Lactas Recens.*—*Retail Chem.*, ii/1932, 271.

Detection of Lactic Acid. A bright red coloration is obtained when 0.2 ml. of solution, containing less than 0.2% of lactic acid, is heated for two minutes at 100° with 2 ml. of conc. H_2SO_4 , cooled and treated with two drops of 5% alcoholic guaiacol solution.

This reaction is not given by formic, acetic, malic, benzoic or salicylic acids. Citric acid gives a yellow colour, tartaric acid a slight red colour, and tannin a blackish-violet.—*J. chem. Soc. Abstr.*, ii/1921, 356.

Calcii et Sodii Lactas (B.P.C. Supp. V).

$C_{12}H_{20}O_{12}CaNa_2 \cdot 4H_2O = 514.3$. Contains 7.5 to 8.5% of Ca and 8.5 to 10% of Na. A free acid limit equivalent to approximately 0.9% of lactic acid is included. Calcium is determined gravimetrically, by precipitation as oxalate and titration with sulphuric acid of the ignited residue; titration of the total alkalinity of the ash, less the titration for calcium, is calculated to Na.

Assay. Moisture.—This is best determined by drying for 2 hours below 100° and subsequently to constant weight at 130°. **Calcium.**—Instead of titrating the ignited oxalate with N/10 sulphuric acid, it is better to dissolve in N/2 hydrochloric acid and back titrate with N/2 sodium hydroxide. **Sodium.**—Dissolve the substance (containing 5 to 10 mg. of sodium) in 5 to 10 ml. of water and for every 3 mg. of sodium anticipated add 3 ml. of Kahane's reagent (magnesium acetate 100 g., uranyl acetate 32 g., glacial acetic acid 20 ml., alcohol (90%) 500 ml., water to 1 litre, heat on a steam-bath until dissolved, cool, filter at 20° and store in an amber-coloured bottle). Cool in ice-cold water for 30 minutes, stirring occasionally. Collect the precipitate in a sintered glass crucible, wash with 5 to 10 ml. of reagent, followed by 10 ml. of alcohol (95%). Dry at 105° to 110°. 1 g. of residue = 0.0153 g. of Na. **Lactic Acid.**—Dissolve 0.35 g. in 20 ml. of water, and add 30 ml. of 10% sulphuric acid and 25 ml. of N/2 potassium dichromate. Reflux for 15 minutes, cool, dilute, add 10 ml. of 10% potassium iodide solution and, after five minutes, titrate with N/10 thiosulphate, a blank being carried out simultaneously.—S. G. Liversedge, *Quart. J. Pharm.*, 1937, 364.

Calcii Lactas (B.P.). $C_6H_{10}O_6Ca \cdot 5H_2O = 308.2$. As indicated by weight of the sulphated residue, it contains from 97% to the equivalent of 103% of the pure substance. The *B.P.*'14 required a purity of only 93% and a test for limit of various sugars was not included. *Calcii Lactas, U.S.P. XII*, dried at 120°, loses 25 to 30% of water and then contains not less than 98% of the anhydrous salt; determined by precipitation with ammonium oxalate, decomposition of the precipitate with sulphuric acid and titration with N/10 potassium permanganate. *Lactate de Calcium, Fr. Cx.* 1937, yields not less than 70% of the anhydrous salt which contains not less than 98% of the pure substance, corresponding to 17.82% CaO. *Calcium lacticum, P. Helv. V.*, yields 43 to

46% of CaSO_4 , corresponding to 68.9 to 73.7% of the anhydrous lactate and loses 27 to 29.5% at 120° to 125° . Calcium lacticum, *P.G. VI*, contains 70.5 to 73% of anhydrous calcium lactate.

An examination of a large number of commercial and specially prepared samples showed that there are two kinds of calcium lactate. One is soluble in 20 parts of water at 23° and the other is soluble in 16 parts of water. Since different methods of manufacture give products of different solubility it is useless to set a single standard for solubility.—N. Glass, *Quart. J. Pharm.*, 1933, 522.

The solubility does not decrease on keeping nor is there any appreciable loss of water of crystallisation. The solubility varies with the method of preparation but does not appear to be due to any variation in constitution.—G. H. Macmorran, *Pharm. J.*, i/1933, 245.

Tabellæ Calcii Lactatis (*N.F. VII*). Contain 92.5 to 107.5% of the labelled amount of calcium lactate, including all tolerances. Assayed by ignition to carbonate, dissolving the residue in dilute hydrochloric acid, filtering and precipitating the calcium in an aliquot of the filtrate with ammonia and ammonium oxalate solution, filtering and washing the precipitate, finally transferring the filter to a flask, adding water and dilute sulphuric acid and titrating with N/10 potassium permanganate.

Ethyl Lactate, $\text{CH}_3\text{CHOH}\cdot\text{COOC}_2\text{H}_5$, a colourless liquid used as a solvent. *B.S.S. No.* 663—1936 includes requirements regarding specific gravity (1.032 to 1.040 at 15°), distillation range (not less than 96% between 135° and 160°), residue, acidity, ester content and miscibility in all proportions with petroleum ether and water.

Ferri Lactas (*B.P.C.*). $\text{C}_6\text{H}_{10}\text{O}_6\text{Fe}\cdot 3\text{H}_2\text{O} = 288.0$. Residue on ignition, 26.5 to 28.5%, being equivalent approximately to a purity of 96%. Tartaric, citric and malic acids are tested for by addition of lead acetate solution. By titration with sodium thio-sulphate of the iodine liberated on the addition of potassium iodide and hydrochloric acid, a ferric iron limit of about 0.8% is specified. The *Fr. Cx.* 1937 substance yields on ignition 27% of Fe_2O_3 .

Sodii Lactas (*B.P. Add. IV*). $\text{C}_3\text{H}_5\text{O}_3\text{Na} = 112.04$. Contains 68 to 72% *w/w* of the pure salt, by ignition and titration of the residue with excess N/2 sulphuric acid and N/2 sodium hydroxide, using methyl orange indicator. Limits of alkalinity and of various sugars are included.

ACIDUM MANDELICUM

Acidum Mandelicum (*B.P. Add. IV and VI*).

$\text{C}_6\text{H}_5\text{CHOH}\cdot\text{COOH} = 152.1$. Contains not less than 99.5% of $\text{C}_6\text{H}_5\text{O}_3$, by titration with N/10 sodium hydroxide to phenolphthalein. *M.p.*, 119° to 121° . Loss at 100° , not more than 0.5%. Ash, not more than 0.1%. A test for limit of chlorinated compounds is included. The *U.S.P. XII* requires Acidum Mandelicum, after drying for 24 hours over sulphuric acid, to contain not less than 99.0% of the pure substance.

Tentative methods for the determination of mandelic acid and its salts in tablets and liquid preparations are described in *Methods of Analysis* (*A.O.A.C.*, 1940, 610). The mandelic acid, extracted with chloroform-ether (2 + 1) from an acidified solution of the sample, is dissolved in water free from carbon dioxide and titrated with standard sodium hydroxide, using phenolphthalein as indicator.

Estimation in Preparations. A convenient method is presented for determining mandelic acid in calcium mandelate, monoethanolamine mandelate

and elixir of mandelic acid, which involves an ether extraction in acid solution and a direct titration by means of N/10 barium hydroxide solution.—A. N. Stevens and E. J. Hughes, *J. Amer. pharm. Ass.*, 1939, 222.

Calcii Mandelas (U.S.P. XII). $\text{Ca}(\text{C}_8\text{H}_7\text{O}_3)_2 = 342.3$. Dried at 110° , loses not more than 1% and then contains 98.5% of $\text{Ca}(\text{C}_8\text{H}_7\text{O}_3)_2$. Tests for completeness of solution in boiling water, free acid, chloride, sulphate and heavy metals are included. Magnesium and alkali salts are determined by dissolving the sample in dilute hydrochloric acid, rendering the solution alkaline with ammonia, precipitating the calcium as oxalate, filtering and evaporating an aliquot of the filtrate with 0.5 ml. of sulphuric acid to dryness. 0.5 g. should yield not more than 10 mg. of residue. Assayed by precipitation with ammonium oxalate, washing the residue, decomposition of the calcium oxalate with dilute sulphuric acid and titration with N/10 potassium permanganate.

ACIDUM NITRICUM

Acidum Nitricum (B.P.). Contains 69 to 71% by weight of HNO_3 . Sp. gr., about 1.42. Acidum Nitricum Dilutum, B.P.C. contains 9.5 to 10.5% w/w of HNO_3 , with sp. gr. 1.054 to 1.060 and is of the same strength as the dilute acid of the B.P. '14. The acid of the U.S.P. XII contains 67 to 71% of HNO_3 . The *Fr. Cx.* 1937 acid contains not less than 63.05% of HNO_3 . Acidum nitricum, P.G. VI, contains 24.8 to 25.2% of HNO_3 . Acidum nitricum crudum 61 to 65% and Acidum nitricum fumans not less than 86%. Acidum nitricum concentratum, P. *Helv.* V, contains 64 to 66% w/w of HNO_3 .

British Standard Density-Composition Tables for Aqueous Solutions of Nitric Acid (B.S.S. No. 975—1941) give the concentration in g. of HNO_3 per 100 g. and in g. of HNO_3 per 1000 ml. corresponding to densities of 1 to 1.531 for each 2° over the range 10° to 40° . The tables are designed for use in conjunction with British Standard Density Hydrometers.

Nitrous Fumes in Industry

Nitrous fumes are usually reddish-brown in colour and may consist chiefly of a mixture of nitrogen peroxide (NO_2) with smaller quantities of nitric oxide (NO). They are formed by the action of concentrated nitric acid on metals or organic matter, such as wooden flooring, in the manufacture of nitric acid and sulphuric acid by the lead chamber process, and in the manufacture of explosives and dyes. In war-time they may cause trouble in confined spaces, such as gunpits, armoured cars and magazines, when combustion is incomplete. They are also liable to occur in mining operations, where explosives are employed. The fumes are exceedingly dangerous on account of the delayed action and workmen may be unaware of any effects but collapse after returning home, oedema of the lungs developing and death occurring in about 24 hours. Exposure to from 2 to 8 parts per 10,000 for a short time may be fatal, even 1 to 1.5 parts per 10,000 for a short exposure being dangerous, whilst 1 part per 100,000 is the maximum concentration allowable for several hours' exposure.

Treatment. Prompt removal to fresh air is essential. The patient should be kept warm in a prone position, and given stimulants, such as tea, but not alcohol. Strict confinement to bed for not less than 2 days is advisable.

Detection and Determination. The official method does not determine actual nitrous fumes, but the quantity of nitrous acid or nitrogen trioxide present in the fumes and is dependent on the well-known Griess-Ilosvay reaction (see this volume, p. 853) for the determination of nitrites in water. For comparison a standard colour solution is prepared by acidifying an acetone solution of dimethylamino-azo-benzene with hydrochloric acid.—(*Toxic Gases in Industry*, No. 5, D.S.I.R.)

Potassii Nitras (B.P.). $\text{KNO}_3 = 101.1$. A purity of 99% is required. The assay process of the B.P. reduces the nitrate with nascent hydrogen obtained by addition of sulphuric acid and reduced iron, liberating the ammonia formed with sodium hydroxide, distilling into excess of standard acid and back titrating with sodium hydroxide; a blank titration is performed. Potassii Nitras, *U.S.P. XII*, contains 99% of KNO_3 after drying to constant weight at 110° ; assayed by evaporation with hydrochloric acid and titration of the chloride with silver nitrate and ammonium thiocyanate.

Uranii Nitras (B.P.C. Supp. IV). $\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O} = 502.2$. Determined by precipitation as hydroxide and ignition to oxide (U_3O_8), it contains not less than 98% of the pure hexahydrate. A 5% solution remains clear on the addition of an equal volume of ammonium carbonate solution (limit of alkaline earths) and does not show more than a faint darkening (due to lead, iron, manganese or zinc) on saturating with hydrogen sulphide. It should contain no more uranous compounds in 1 g. dissolved in 20 ml. water acidified with dilute sulphuric acid, than will decolorise 0.1 ml. of N/10 potassium permanganate.

Determination of Nitrates. Nitrates can be determined by using Nitron, *Syn.* 1 : 4-Diphenyl-3 : 5-endanilo-4 : 5-dihydro-1 : 2 : 4-triazole. $\text{C}_6\text{H}_5 \cdot \text{N} \cdot \text{N} : \text{C} \cdot \text{N}(\text{C}_6\text{H}_5) \cdot \text{CH} \cdot \text{N} \cdot \text{C}_6\text{H}_5$. The reagent is used as a 10% solution in N/1 acetic acid. To 100 ml. of aqueous solution containing 0.1 g. of the nitrate acidified with acetic acid and heated to boiling, is added 10 ml. of the reagent. The mixture is cooled at 0° for 2 hours and the precipitate then collected, washed with a saturated aqueous solution of nitron, dried at 110° and weighed. Each gramme of precipitate is equivalent to 0.1653 g. of NO_3 . Bromides, iodides, nitrites and chromates should be removed before the reagent is added.

Sodii Nitris (B.P.). $\text{NaNO}_2 = 69.005$. Estimated by titration of standard potassium permanganate, acidified with sulphuric acid, diluted, and warmed to 40° with a 0.5% solution of the salt. 95% purity is required. Sodii Nitris, *U.S.P. XII*, contains 97% after drying over sulphuric acid. A solution of the salt is added to excess acidified permanganate, warmed to 40° , stood for 5 minutes and then excess standard oxalic acid added, heated to 80° , and back titrated with permanganate. The *Fr. Cx.* 1937 requires the same purity.

Tabellæ Sodii Nitritis (*U.S.P. XII*). Contain 93 to 107% of the labelled amount of NaNO_2 , including all tolerances. Assayed by dissolving a weighed quantity in water, filtering, making up the filtrate to volume, adding saturated potassium chlorate solution and N/10 silver nitrate followed by nitric acid to an aliquot of the solution, filtering and titrating the excess of N/10 silver nitrate in an aliquot of the filtrate with N/10 ammonium thiocyanate. If more than 0.1% of Cl is present, it is determined by treating the same volume of tablet solution with N/10 silver nitrate and nitric acid, heating until no more nitrous fumes are evolved, filtering and titrating an aliquot of the filtrate with N/10 ammonium thiocyanate. The volume of N/10 silver nitrate required by the Cl present is then deducted from the volume consumed in the assay.

Liquor Æthylis Nitritis Concentratus (*B.P. Add. V*). Contains, when freshly prepared, 17.0 to 20% *w/v* of $\text{C}_2\text{H}_5\text{O}_2\text{N}$, or after storage or when the container has been opened occasionally, not less than 10% of $\text{C}_2\text{H}_5\text{O}_2\text{N}$; determined by the process described under *Spiritus Ætheris Nitrosi*.

Spiritus Ætheris Nitrosi (*B.P.*). Contains from 1.25 to 2.5% *w/v* of ethyl nitrite, approximately the same as the *B.P.* '14 preparation which was required to contain 1.52 to 2.66% *w/w*. Sp. gr., 0.838 to 0.842. There is also a test for limit of acid. Assayed by the *B.P.* process in which the ethyl nitrite is decomposed by shaking in a brine-charged nitrometer with potassium iodide solution and dilute sulphuric acid, the liberated nitric oxide being measured. Each ml. of moist nitric oxide is equivalent to 0.0032 g. of $\text{C}_2\text{H}_5\text{O}_2\text{N}$. *Spiritus Æthylis Nitritis, N.F. VII*, contains 3.5 to 4.5% *w/w* of $\text{C}_2\text{H}_5\text{ONO}$; determined by passing carbon dioxide through a cooled solution of potassium iodide, deaerated water, and dilute hydrochloric acid, decolorising if necessary with N/10 sodium thiosulphate, adding the spirit just above the surface of the liquid and immediately titrating with N/10 sodium thiosulphate; both the pipette and tip of the burette are introduced into the flask through an outlet tube. *Spiritus Ætheris nitrosi, P. Helv. V*, is a solution in absolute alcohol containing from 2 to 2.5% *w/v* of ethyl nitrite. It is assayed as follows:—Shake vigorously for 5 minutes in a closed flask 20 ml. of N/10 silver nitrate, 10 ml. of the spirit, 20 ml. of saturated solution of potassium chlorate and 5 ml. of dilute nitric acid, and then dilute to 100 ml. with water. Filter and titrate 50 ml. of the filtrate with N/10 ammonium thiocyanate using iron alum as indicator; each ml. of N/10 AgNO_3 used up is equivalent to 0.022515 g. of $\text{C}_2\text{H}_5\text{O}_2\text{N}$.

This preparation decomposes even when kept under the best conditions. The first change is probably the formation of aldehyde and nitrous acid, and then the aldehyde is oxidised into acetic acid. In the course of time the nitrous constituent of the spirit entirely disappears, but aldehyde, one of the most readily oxidisable bodies, remains. The spirit should be kept in cool cupboards and in well-filled bottles, preferably upside down.

With regard to the volatilisation of ethyl nitrite, Cowley has shown that every trace of ethyl nitrite disappears from a solution within a few days in ordinary vessels. As to decomposition in an aqueous solution, a mixture containing spirit of nitre loses the whole of it in three days. Solutions in absolute alcohol change less rapidly than those in 90% alcohol on account of the water present. A mixture of 90% alcohol and glycerin in equal volumes is a good solvent for all preparations of ethyl nitrite.

ACIDUM OLEICUM

Acidum Oleicum (B.P.). Consists chiefly of $C_{17}H_{33}COOH$. Sp. gr., about 0.898. Acid value, 195 to 200. Iodine value, 85 to 90. It should not become cloudy on cooling until a temperature of 10° has been reached; a higher point indicates the presence of stearic acid; it congeals at about 4° . Should comply with limits for mineral acids and for neutral fats and mineral oils. Ash not more than 0.1% w/w. The *U.S.P. XII* substance has a congealing temperature not above 10° ; acid value, 188 to 203; iodine value, 85 to 95. Acidum oleicum, *P. Helv. V*, has an acid value of 195 to 205 and an iodine value, 78 to 90.

Acidum Ricinoleicum (B.P. Add. VI). Sp. gr., 0.945 to 0.948. Acid value, not less than 175. Iodine value, 85 to 91. n_D at 40° , 1.462 to 1.468.

Acidum Stearicum (B.P.C.). Should not melt below 54° . Acid value, 200 to 210. Complies with a limit test for neutral fat or paraffin. Acidum Stearicum, *U.S.P. XII*, has a congealing point not below 54° . Iodine value, not more than 4.

Alcohol Cetylicum (B.P.C. Supp. III). M.p., 48° to 52° . Ash, not more than 0.1%. Iodine value, not more than 20. Tests for limit of paraffin and of fatty acids are included.

ACIDUM PHOSPHORICUM

Acidum Phosphoricum (B.P.). Contains 88 to 90% w/w of H_3PO_4 with a sp. gr. of about 1.75. The corresponding acid of the 1914 Pharmacopœia contained only 66.3% with sp. gr. 1.5. No brown colour, indicative of phosphorous and hypophosphorous acids, is produced on warming with silver nitrate. Acidum Phosphoricum Dilutum, *B.P.*, contains 9.5 to 10.5% w/w of H_3PO_4 . Assayed in saturated salt solution by titration with N/1 sodium hydroxide, using phenolphthalein as indicator; each millilitre of normal solution is equivalent to 0.04902 g. of H_3PO_4 . The corresponding acids of the *U.S.P. XII* contain 85 to 88% w/w and 9.5 to 10.5% w/v of H_3PO_4 respectively. Tests for phosphate, in which no turbidity should be produced with ether (6 parts) and alcohol (2 parts), and for nitrate by treatment with sulphuric acid and indigo solution, are included. Acide Phosphorique Officinal, *Fr. Cx. 1937*, determined by precipitation with magnesia mixture and ignition to pyrophosphate, contains not less than 49.7% of H_3PO_4 . The dilute acid contains 10% H_3PO_4 . Acidum phosphoricum, *P.G. VI*, contains 24.8 to 25.2% of H_3PO_4 .

The best method for the complete titration of P_2O_5 , NaH_2PO_4 and Na_2HPO_4 is the use of phenolphthalein at a temperature of 55° to 70° after $CaCl_2$ has been added to the phosphate solution. The proportion of phosphate to $CaCl_2$ should be 1 in 2 to 1 in 5.—*per Yearb. Pharm.*, 1926, 305.

Metaphosphoric Acid, $HPO_3=80.0$, is equivalent to *glacial phosphoric acid*, and is employed as an albumin test (*vide Urine*, p. 641).

Ammonii Phosphas (B.P.C.). A mixture of the di-ammonium and di-hydrogen salts, containing not less than 20% of NH_3 . The B.P.C. directs 1 g. to be dissolved in 200 ml. of water, 25 ml. of 25% sodium hydroxide solution added, the liberated ammonia distilled into 50 ml. of N/2 sulphuric acid and the excess acid titrated with N/2 sodium hydroxide to methyl red.

Calcii Phosphas (B.P.). Assayed by addition of ammonium acetate and ammonium oxalate to a solution slightly acidified with hydrochloric acid, heating and allowing to stand for several hours, filtration and ignition with sulphuric acid of the washed precipitate. Contains calcium equivalent to 85% of $\text{Ca}_3(\text{PO}_4)_2$. Calcii Phosphas Tribasicus, U.S.P. XII, loses not more than 8% on ignition, and then contains not less than 90% of $\text{Ca}_3(\text{PO}_4)_2$. Determined by precipitation with ammonium molybdate solution, decomposition of the washed precipitate with N/1 sodium hydroxide and back titration with N/2 sulphuric acid, using phenolphthalein indicator. Limit of fluorine, not more than 0.005%; 2 g. of the substance, 5 ml. of perchloric acid and 15 ml. of distilled water is distilled at 135° to 145° , by gradual additions of water, until 70 ml. has been collected. An aliquot is adjusted to the same reaction to sodium alizarinsulphonate as distilled water by addition of N/20 sodium hydroxide. 1 ml. of N/10 hydrochloric acid is added to the solution and to the water control, and 0.025% thorium nitrate is added gradually to the test solution until the liquid is faintly pink and the same quantity added to the water control. Sodium fluoride solution (0.00222%) is then added to the control until after adjusting to the same volume, the colours match. By weight of magnesium pyrophosphate the *Fr. Cx.* 1937 requires Phosphate Di-acide de Calcium to contain 97% of the monohydrate, Phosphate Mono-acide de Calcium to contain 97% of the dihydrate, and Phosphate Neutre de Calcium, determined on the substance dried at 100° , to contain 92% of the anhydrous substance; loss of the latter at 100° , not more than 3% and on incineration not more than a further 5%. Calcium phosphoricum, *P.G. VI*, leaves 25 to 26.2% of residue on ignition. Calcium phosphoricum bibasicum, *P. Helv. V*, is determined by dissolving 1 g. in 25 ml. of N/1 hydrochloric acid, diluting with 150 ml. of water and titrating to methyl orange with N/1 sodium hydroxide; 1 ml. is equivalent to 0.1361 g. of CaHPO_4 and the salt should contain from 78.8 to 80.2% of anhydrous CaHPO_4 . Mono- and tribasic salts are excluded by titration to phenolphthalein after the previously neutralised solution has been treated with neutral calcium chloride solution. The same salt for veterinary use must contain from 35 to 38% of P_2O_5 . Calcium phosphoricum monobasicum, *P. Helv. V*, contains not more than 5% of free phosphoric acid and not less than 92% of $\text{Ca}(\text{H}_2\text{PO}_4)_2 + \text{H}_2\text{O}$. Phosphoric acid determined by titration to methyl orange with N/1 sodium hydroxide and $\text{Ca}(\text{H}_2\text{PO}_4)_2$ by continuing the titration to phenolphthalein with addition of calcium chloride, calculating by subtracting twice the methyl orange reading from the phenolphthalein reading. Calcium

phosphoricum tribasicum, *P. Helv. V*, is assayed by dissolving about 1 g., accurately weighed, in 25 ml. of N/1 hydrochloric acid, diluting with 200 ml. of water and, after adding 3 or 4 drops of methyl orange, titrating with N/1 sodium hydroxide until the red colour disappears, rotating constantly to redissolve the precipitate formed. Each ml. of N/1 HCl is equivalent to 0.07756 g. of $\text{Ca}_3(\text{PO}_4)_2$ and the salt should contain not less than 95%.

Determination of Phosphorus in Syrups. An amount of material corresponding to from 15 to 20 mg. of P_2O_5 is dissolved in 70 ml. of water, 5 ml. of nitric acid is added and 10 g. of ammonium nitrate, and the temperature raised to 65°. To the stirred solution is then added, in a thin stream, 35 ml. of nitric acid solution of ammonium molybdate. The mixture is stirred for a further 30 seconds, allowed to stand at a temperature of 65° to 70° for 15 minutes, removed from the water-bath and allowed to cool for a further 15 minutes, the supernatant liquid being then filtered off through an asbestos or paper pulp filter in a Gooch crucible under slight suction. The precipitate is washed, as far as possible by decantation, with two 20 ml. portions of 5% nitric acid, then with five 20 ml. portions of 5% ammonium nitrate solution, and finally with small quantities of water. The final washings should not redden litmus paper. The precipitate is transferred to the original beaker, the crucible being washed out with about 50 ml. of water, 50 ml. of N/5 sodium hydroxide (free from carbonate) is added, the yellow precipitate allowed to dissolve completely, and the excess of alkali titrated with N/5 hydrochloric acid, using thymol blue or phenol violet as indicator and titrating to the yellowish-green tint.—G. J. W. Ferrey, *Quart. J. Pharm.*, 1934, 347.

Magnesii Phosphas (B.P.C. Supp. IV). Consists of $\text{Mg}_3(\text{PO}_4)_2$ with about 30% of combined water. The residue on ignition is not less than 68%. **Magnesii Phosphas Tribasicus, U.S.P. XII**, loses on ignition, 20 to 27% of its weight and then contains not less than 98% of $\text{Mg}_3(\text{PO}_4)_2$. Assayed by the method described for **Calcii Phosphas Tribasicus**. Limits for carbonate, residue insoluble in hydrochloric acid (0.2%), barium, heavy metals, arsenic, soluble salts (1.5%), sulphate, chloride, nitrate, calcium and of dibasic salt and magnesium oxide are included.

Tabellæ Magnesii Phosphatis Tribasici, (U.S.P. XII). Contain 94 to 108% of the labelled amount of $\text{Mg}_3(\text{PO}_4)_2 \cdot 5\text{H}_2\text{O}$, including all tolerances. Limit tests for calcium and soluble salts are included. Assayed by igniting a weighed quantity of powdered tablets, dissolving the residue in nitric acid and water, filtering and thoroughly washing the filter with hot water. After the filtrate has been made slightly alkaline with ammonia solution, the precipitate formed is dissolved by addition of nitric acid and the solution warmed to 50° and solution of ammonium molybdate added. The mixture is kept at 50° for 30 minutes and the precipitate then washed with cold water until free of acid. The precipitate is dissolved in a known excess of N/1 sodium hydroxide and the excess alkali titrated with N/2 sulphuric acid, using phenolphthalein indicator.

Potassii Phosphas (B.P.C.). $\text{K}_2\text{HPO}_4 = 174.2$. Loses not more than 5% at 100° and then contains 98% of the pure substance. Assayed by titration with N/2 sulphuric acid to the green colour of bromocresol green indicative of pH 4.5, which converts the dipotassium compound into the di-hydrogen compound.

Potassii Phosphas Acidus (B.P.C.). $\text{KH}_2\text{PO}_4 = 136.1$. Contains 97% of KH_2PO_4 . Titrated with standard sodium hydroxide in the presence of sodium chloride using phenolphthalein as indicator. A limit of dipotassium phosphate by neutralisation with N/10 sulphuric acid to the pH 4.5 green colour

of bromocresol green, equivalent to about 1.7% of K_2HPO_4 , is included.

Sodii Phosphas (*B.P. Add. I*). $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O} = 358.2$. Assayed by titration to pH 4.5 with N/2 sulphuric acid using bromocresol green as indicator, which replaces the *B.P.* '14 titration to NaH_2PO_4 with methyl orange indicator. Contains from 99% to the equivalent of 105% of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$. A test for absence of fluorides, by treatment of a solution acidified with acetic acid, with calcium chloride solution is included; no turbidity should be produced on standing for 1 hour. Sodii Phosphas, *U.S.P. XII*, loses 43 to 50% at 110° , and then contains not less than 98% of Na_2HPO_4 ; assayed by precipitation with magnesia mixture, finally weighing the ignited residue of pyrophosphate.

Sodium phosphate may contain fluoride as impurity, which may cause serious disturbance in biochemical work.—A. Harden, *Nature, Lond.*, ii/1934, 101.

The addition of 10 ml. of a 33% solution of sodium hexametaphosphate (Calgon) to the Fehling's solution immediately before the addition of the invert sugar will eliminate interference due to the presence of calcium. When an excess of sodium hexametaphosphate is added to a solution of a calcium salt the calcium becomes inert and cannot be detected by any of the normal reagents.—J. G. W. Gaskin, *Analyst*, 1935, 318.

Sodii Phosphas Acidus (*B.P.*). $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O} = 156.1$. Contains not less than 98% of the pure salt. It is assayed by titration with N/2 sodium hydroxide after the addition of 25% of sodium chloride. A titration of not more than 1 ml. of N/10 sulphuric acid for 1 g. of salt, to bromocresol green, represents a limit of the di-sodium salt equivalent to about 1.4% of Na_2HPO_4 . The *U.S.P. XII* requires Sodii Biphosphas to lose 10 to 15% of water when dried for 1 hour at 60° and then at 100° ; it then contains 98% of the anhydrous acid phosphate; the acidity or alkalinity (disodium phosphate) to methyl orange of 2 g. is between 0.3 ml. N/1 sodium hydroxide and 0.3 ml. N/1 sulphuric acid. Phosphate Monoacide de Sodium, *Fr. Cx.* 1937, contains not less than 99% of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$. The anhydrous salt contains not less than 98%.

Sodii Phosphas Exsiccatus (*B.P.C.*). Loses not more than 2% at 100° and then contains 99% of Na_2HPO_4 , determined by the *B.P.* method for Sodii Phosphas. Sodii Phosphas Exsiccatus, *U.S.P. XII*, contains 98% of the anhydrous compound after drying to constant weight at 110° ; loss at 110° , not more than 5%; assayed by precipitation as magnesium ammonium compound and ignition to pyrophosphate.

Sodium Acid Pyrophosphate, as used in baking powder, is the anhydrous compound, $\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$. The aqueous solution is neutral to methyl orange and acid to phenolphthalein.

Estimation of Phosphates. The following modified procedure for the colorimetric determination of phosphates is recommended:—*Reagents.* Ammonium molybdate solution, 5 g. of ammonium molybdate in 100 ml. of N sulphuric acid.

Hydroquinone solution, 0.5 g. of hydroquinone dissolved in 100 ml. of distilled water made slightly acid with one drop of concentrated sulphuric acid.

Sodium succinate solution, 20 g. (anhydrous basis) of sodium succinate in 100 ml. of distilled water. *Sample.* All phosphorus should be in the ortho

form of the acid in clear colourless solution at about pH 5.0. *Procedure.* To an aliquot of the sample equivalent to not more than 0.3 mg. phosphorus and contained in a volume of not less than 10 ml., add the following in order, mixing well after each addition: (1) 2 ml. ammonium molybdate solution, (2) 2 ml. hydroquinone solution, (3) 2.5 ml. sodium succinate solution. Adjust the volume to 25 ml. with distilled water and measure the colour at 460 m μ within four hours.—L. S. Stoloff, *Industr. Engng Chem. (anal. Edn.)*, 1942, 637.

ACIDUM SALICYLICUM

Acidum Salicylicum (B.P.). $C_6H_4OH \cdot COOH = 138.0$. M.p., 158° to 159°. When titrated in alcoholic solution with N/2 sodium hydroxide using phenol red as indicator a purity of 99.5% is indicated. Ash not more than 0.05%. No assay was included in the 1914 Pharmacopœia. The *U.S.P. XII* requires the same purity after drying the acid for 5 hours over sulphuric acid; titration of a solution in alcohol is made with N/10 sodium hydroxide using phenolphthalein as indicator. Acidum salicylicum, *P. Helv. V*, is required to contain at least 99.6% of the pure acid and to melt between 155° and 157°.

Vanadate Test for Salicylic Acid. Mix equal parts by volume of 40% formaldehyde and conc. sulphuric acid and cool the mixture thoroughly. Moisten the substance to be tested in a dish with the mixture, add a little ammonium vanadate and stir well. If salicylic acid is present a prussian blue colour appears immediately, changing rapidly to greenish blue and finally green. For about 1 mg. of salicylic acid use 2 drops of the liquid and 2 to 3 mg. of ammonium vanadate. The only other substance giving the colour is salicylic aldehyde.—*Pharm. J.*, i/1915, 521.

The second report of the Commission Internationale des Spécialités includes an agreed method for the determination of salicylic acid in which the acid is converted to tribromophenol by the addition of excess of bromide-bromate solution and acidifying, the excess of bromine being determined iodometrically.—*Bull. Fed. int. Pharm.*, 1938, 76.

Assay of Ointment. The following assay for salicylic acid in the *B.P.* 1932 ointment is recommended by the Sub-Committee on the Assay of Galenicals of the Committee on Pharmaceutical Chemistry:—Boil about 5 g., accurately weighed, with 20 ml. of water and 20 ml. of N/1 sodium hydroxide under a reflux condenser for fifteen minutes. Filter through a wet filter paper, wash the residue and filter with hot water; cool, and dilute the mixed filtrate and washings to 100 ml. Transfer 50 ml. to a stoppered flask; add 50 ml. of N/10 bromine, 50 ml. of water and 5 ml. of hydrochloric acid, shake repeatedly during half an hour, add 15 ml. solution of potassium iodide and titrate with N/10 sodium thiosulphate. Each ml. of N/10 bromine is equivalent to 0.00230 g. of $C_6H_4O_2$. Limits of 1.9 to 2.1% of $C_6H_4O_2$ are suggested. (*British Pharmacopœia Commission Report*, No. 11, May 1939.)

The use of salicylic acid as a preservative is now illegal (see p. 837).

Detection. Concentrate liquid (distil off any alcohol) in presence of alkali and sodium chloride, acidify and shake out with chloroform, evaporate and add ferric chloride solution—violet colour. See also Scheme for Recognition of Organic Chemicals and under Acidum Benzoicum, p. 6.

Official quantitative and qualitative methods for the determination of salicylic acid in foods are described in *Methods of Analysis (A.O.A.C., 1940, 454)*.

Detection and Determination in Presence of Benzoic Acid. Nicholls's modification of the Jorissen test is advocated. To the solution to be tested, containing about 20 mg. of mixed acids in 25 ml., is added 1 ml. of 2% sodium nitrite solution and 1 ml. of 0.3% crystalline copper sulphate solution in 10% acetic acid. The mixture is heated in a boiling water-bath for 15 minutes, cooled and diluted to 50 ml. The red colour is matched against that given by a standard solution prepared by carrying out the test on 5 ml. of 0.1% salicylic

acid solution diluted to 40 ml., using 5 ml. each of the nitrite and copper sulphate solutions and diluting the final solution to 100 ml.

The benzoic acid may be determined after destroying the salicylic acid with alkaline potassium permanganate. The solution is warmed to 50° with 5 ml. of N/10 potassium hydroxide and N/10 permanganate added slowly until a pink colour persists. The mixture is then acidified with 1 in 3 dilute sulphuric acid and decolorised with acetic acid added drop by drop. The benzoic acid is extracted with ether from the solution saturated with ammonium sulphate, converted to the ammonium salt and determined by the method for foodstuffs (see p. 6). The method can be applied to the determination of sodium salicylate and sodium benzoate in compound glycerin of thymol by separating the mixed acids by acidification and extraction with ether following a preliminary extraction of the volatile oils from an unacidified 1 in 5 solution by means of ether.—F. W. Edwards, H. R. Nanji and M. K. Hassan, *Analyst*, 1937, 175.

A tentative method for the determination of salicylic acid in the presence of other phenols is described in *Methods of Analysis (A.O.A.C., 1940, 572)*.

Ammonii Salicylas (N.F. VII). $\text{NH}_4\text{C}_7\text{H}_5\text{O}_3 = 155.1$. After drying for 24 hours over sulphuric acid it contains not less than 98% of the pure substance; assayed by the method described for Sodium Salicylate, *B.P.*, using tenth-normal hydrochloric acid.

Magnesium Salicylas (B.P.C.). $\text{C}_{14}\text{H}_{10}\text{O}_6\text{Mg} \cdot 4\text{H}_2\text{O}$. Loses not more than 19% at 110° and then contains 99% of the anhydrous salt. Assayed by the *B.P.* process for Sodii Salicylas by titration with standard sulphuric acid to bromophenol blue.

Potassii Salicylas (B.P.C.). $\text{C}_7\text{H}_5\text{O}_3\text{K} = 176.1$. Dried at 110° it contains 99% of the pure substance. Moisture, determined at 110°, not more than 1%. Assayed by the *B.P.* ether-acid titration used for Sodii Salicylas.

Sodii Salicylas (B.P.). $\text{C}_6\text{H}_4\text{OH} \cdot \text{COONa} = 160.0$. A purity of 99.5% is required for the substance dried at 110°. Loss at 110° not more than 1%. The separated salicylic acid melts between 158° and 159°. It is assayed by titration with half normal sulphuric acid, removing the liberated acid with ether and using bromophenol blue as indicator. Sodii Salicylas, *U.S.P. XII*, after drying for 4 hours at 110°, contains 99.5% of $\text{NaC}_7\text{H}_5\text{O}_3$. Limit of moisture 0.5%; assayed similarly using N/2 hydrochloric acid. Salicylate de Sodium, *Fr. Cx.* 1937, assayed as the lithium salt, contains 99% of the sodium salt, corresponding to 85.4% of salicylic acid.

Tabellæ Sodii Salicylatis (U.S.P. XII). Contain 93 to 107% of the labelled amount of $\text{NaC}_7\text{H}_5\text{O}_3$, including all tolerances. Assayed by transferring a weighed quantity of powdered tablets to a separator with water, acidifying and extracting the liberated salicylic acid with ether, removing the ether, dissolving the residue in alcohol and water and titrating with N/10 sodium hydroxide, using phenolphthalein indicator.

Strontii Salicylas (N.F. VII). $(\text{C}_6\text{H}_4\text{OH} \cdot \text{COO})_2\text{Sr} \cdot 2\text{H}_2\text{O} = 397.7$. Loses not more than 3% over sulphuric acid for 2 hours, and then contains not less than 99% of the pure salt; assayed by titration with N/2 hydrochloric acid to methyl orange, removing the liberated salicylic acid with ether.

Methylis Salicylas (B.P. Add. I). $\text{C}_8\text{H}_8\text{O}_3 = 152.1$. Assayed for esters by saponification of the neutralised substance with N/2 alcoholic potash during one hour, dilution, and back titration with standard sulphuric acid to phenolphthalein, the equivalent of the titration for free acid being subsequently deducted. A blank titration on the alcoholic potash is performed; each millilitre of

N/2 alcoholic potash is equivalent to 0.07603 g. of $C_6H_5O_3$; a titration difference indicating not less than 98% should be obtained. Sp. gr., 1.186 to 1.191; refractive index at 20° , 1.536 to 1.583. Methylis Salicylas, *U.S.P. XII*, may be the synthetic or the natural substance and must be labelled accordingly. In assaying, the saponification is allowed to proceed for 2 hours; a purity of 98% is required.

Oleum Betulæ (B.P.C.). This naturally occurring oil should contain 98% *w/w* of esters calculated as methyl salicylate and determined by the *B.P.* ester process. Sp. gr., 1.182 to 1.192; optical rotation, $+0.5^\circ$ to -0.5° ; refractive index at 20° , 1.534 to 1.538.

Distinction from Methyl Salicylate. To 5 drops of the oil in a test tube add 5 drops of a 5% alcoholic solution of vanillin and 1 ml. of alcohol, shake well and add 2 ml. of concentrated sulphuric acid; mix thoroughly. Sweet birch oil gives a red coloration while methyl salicylate gives a yellow.—*Perfum. essent. Oil Rec.*, 1914, 60.

Salol (B.P.C.). $C_6H_4 \cdot OH \cdot COOC_6H_5$. The *B.P.C.* requires the substance to melt between 42° and 43.5° . Phenylis Salicylas, *U.S.P. XII*, melts between 41° and 43° .

Tabellæ Phenylis Salicylatis (N.F. VII). Contain 91 to 109% of the labelled amount of phenyl salicylate, including all tolerances. Comply with a test for absence of uncombined phenol and salicylic acid. Assayed by heating with sodium hydroxide solution for 45 minutes over a steam-bath, acidifying the solution and extracting the salicylic acid with ether, washing the ether extracts, evaporating the ether and finally titrating the residue, dissolved in neutral alcohol, with N/10 sodium hydroxide, using phenolphthalein indicator.

Tentative methods for the determination of mixtures of salol and phenacetin, and salol, phenacetin and acetylsalicylic acid are given in *Methods of Analysis (A.O.A.C., 1940)*.

Methylis Hydroxybenzoas (B.P.C. Supp. III). $C_6H_5O_3 = 152.1$. M.p., 122° to 130° . Ash not more than 0.1%. Methylis Parahydroxybenzoas, *N.F. VII*, m.p., 125° to 127° , when dried at 100° and assayed by hydrolysis with N sodium hydroxide and back titration with N sulphuric acid to pH 6.7, contains not less than 99% of $C_6H_5O_3$.

Propylis Hydroxybenzoas (B.P.C. Supp. III). $C_{10}H_{12}O_3 = 180.1$. M.p., 96° to 97° . Ash not more than 0.1%.

Methods are given for the detection and determination of *p*-hydroxybenzoic acid and its esters, cinnamic acid and anisic acid.—F. C. M. Jansen, *Chem. Weekbl.*, 1936, 33, 1.

Detection and Determination in Presence of Salicylic and Benzoic Acids.

Detection. The preservative is extracted from food products, etc., by means of an organic solvent and is hydrolysed with alcoholic potash to decompose any esters of *p*-hydroxybenzoic acid present. The acids are converted to the ammonium salts and the solution boiled until the vapour is neutral. The following tests are then applied to the solution:—(I) Millon's Reagent. (II) Iron Reagent B (see Determination of Benzoic Acid in Foodstuffs, see p. 6). (III) Jorissen's test (see p. 36). (IV) Copper sulphate—evaporate an aliquot part of the ammonium salt solution containing at least 2 mg. of the *p*-acid with 1 ml. of 2% copper sulphate solution on a water-bath to 1–2 ml. (V) Nicholls's benzoic acid test after oxidation of salicylic acid, if present, with alkaline permanganate (see p. 37). (VI) Mohler's test:—A portion of the ammonium salt solution is evaporated to dryness with sufficient N/10 potassium hydroxide

to form the potassium salt and the residue treated as described by Illing (*Analyst* 1927, 225). The following table gives the indications shown by these tests:—

| Test | Result | Inference | Remarks |
|---------------------------------|--|---|------------------------------------|
| I Millon .. | No red colour .. | Salicylic and <i>p</i> -acids absent | Omit tests II, III and IV. |
| | Red colour .. | Salicylic and/or <i>p</i> -acid present | Apply tests II, III and IV. |
| II Ferric chloride .. | Violet colour .. | (a) Salicylic acid present | Confirm by test III. |
| | | (b) <i>p</i> -Acid may also be present | In either case confirm by test IV. |
| | No violet colour | (a) <i>p</i> -Acid alone may be present | |
| | | (b) Salicylic acid absent | Omit test III. |
| III Jorissen .. | Pink colour .. | Confirms presence of salicylic acid. | |
| IV Copper salt | Characteristic crystalline precipitate | Confirms presence of <i>p</i> -acid. | |
| | No crystals within 12 hours | <i>p</i> -Acid absent. | |
| V Nicholls* .. | No violet colour | Benzoic acid absent. | Confirm by test VI. |
| | Violet colour .. | Benzoic acid present. | |
| VI Mohler as modified by Illing | Red colour .. | Confirms presence of benzoic acid. | |

Determination. In the absence of salicylic acid the *p*-acid is determined colorimetrically with Millon's reagent. 20 ml. of ammonium salt solution containing not more than 2 mg. of acid is heated with 2 ml. of the reagent. Different quantities of a standard solution of the *p*-acid are treated similarly and both test solution and the standard are heated in a boiling water-bath for 2 minutes, then diluted to 50 ml. in Nessler glasses and the colours compared. In the presence of salicylic acid, the latter is determined colorimetrically (*i.e.*, by test II or III above). A quantity of the ammonium salt solution containing, say, 0.5 mg. of salicylic acid is diluted to 20 ml. and treated with Millon's reagent as described, standards being prepared with the same weight of salicylic acid and varying quantities of the *p*-acid.—F. W. Edwards, H. R. Nanji and M. K. Hassan, *Analyst*, 1937, 178.

Colour Test. The mixed mono-, bis-, and tris-azophenols produced by coupling *p*-hydroxybenzoic acid with benzene diazonium chloride are insoluble in sodium carbonate solution while the product from salicylic acid is readily soluble. The ethereal solution of the compounds from *p*-hydroxybenzoic acid acquires a red colour when shaken with sodium hydroxide solution. The test is conducted as follows:—The diazotising solution is prepared by adding a 22.5% *w/v* aqueous solution of sodium nitrite to a solution of aniline 5 g. in hydrochloric acid 13 ml. and water 26 ml., the temperature being kept below 5°, until free nitrous acid is present as indicated by starch iodide paper. A small quantity of the mixture of acids is dissolved in sodium hydroxide solution and cooled below 5°. Excess of the diazo solution is added and after a short time the mixture is acidified and extracted with ether. The ethereal extract is then shaken with sodium carbonate to remove the salicylic acid derivative. On shaking with sodium hydroxide the ethereal layer acquires a red colour. If the acids are extracted from foodstuffs they will be obtained in the form of esters which must be hydrolysed before the test is applied.—S. G. Stevenson and J. C. L. Resuggan, *Analyst*, 1938, 152.

ACIDUM SULPHURICUM

Acidum Sulphuricum (B.P.). Contains not less than 95% *w/w* of H_2SO_4 with a sp. gr. of about 1.84. The B.P.

* If tests II and III are positive, the preliminary oxidation with alkaline permanganate must be carried out before applying this test.

limits the amount of oxidisable impurities present by addition of 0.1 ml. of N/10 potassium permanganate to a cooled mixture of 5 ml. with 20 ml. of water, which should not be decolorised within 5 minutes; and nitrate by 5 ml. with 5 ml. of water not decolorising 0.5 ml. of indigo carmine solution. Acidum Sulphuricum Dilutum, *B.P.*, contains from 9.5 to 10.5% *w/w* of H_2SO_4 , and has a sp. gr. of 1.064 to 1.073. The strong acid of the *U.S.P. XII* contains 94 to 98% *w/w* of H_2SO_4 ; sp. gr. at 25°, about 1.84. The dilute acid contains 9.5 to 10.5% *w/v* of H_2SO_4 ; sp. gr. at 25° about 1.064. The acid of the *Fr. Cx. 1937* contains not less than 95% of H_2SO_4 and has a sp. gr. at 20° of about 1.8385. The dilute acid (sp. gr. at 15°, 1.068) contains 10% H_2SO_4 . Acidum sulfuricum, *P.G. VI*, contains 94 to 98%, Acidum sulfuricum crudum not less than 94%, and Acidum sulfuricum dilutum from 15.6 to 16.3% of H_2SO_4 .

The British Standards Institution has published British Standard Density-Composition Tables for Aqueous Solutions of Sulphuric Acid (*B.S.S. No. 753—1937*) giving the concentration of acid in g. of H_2SO_4 per 100 g. and in g. of H_2SO_4 per 1000 ml. corresponding to densities of 0 to 1.834 for each 2° over the range of 10° to 40°. The tables are designed for use in conjunction with British Standard Density Hydrometers.

Acidum Sulphuricum Aromaticum (*B.P.C.*). Contains 12.2 to 13.5% *w/v* of free and combined sulphuric acid. Assayed by evaporation with excess standard sodium hydroxide solution followed by titration of the dissolved residue with standard sulphuric acid, using methyl orange indicator. The *U.S.P. XI* preparation contained free sulphuric acid and ethylsulphuric acid equivalent to 19 to 21% *w/v* of H_2SO_4 .

Calcii Sulphas Exsiccatus (*B.P.C.*). $\text{CaSO}_4, \frac{1}{2}\text{H}_2\text{O} = 145.1$. The *B.P.C.* specifies the solidifying power; a mixture of 20 g. with 10 ml. of water at 15° to 20° sets in about 3 minutes, the test being performed in a cylindrical container of about one inch diameter; after 3 hours the edges of the moulded mass retain their sharpness of outline and do not crumble under pressure of the fingers.

Ligamentum Calcii Sulphatis (*B.P.C.*). The weight of a length of 4 yards of 2-inch bandage is not less than 2.5 ounces, the fabric alone weighing not less than 85 grains; the other widths should be in proportion; not less than an average of 33 threads in the warp and 19 in the weft, per inch. The bandage should be reasonably free from loose powder and 75% of the weight of the bandage should be plaster of paris which is adherent to the fabric.

Magnesii Sulphas (*B.P.*). $\text{MgSO}_4, 7\text{H}_2\text{O} = 246.5$. The 97.4% standard of the 1914 Pharmacopœia is replaced by a standard of not less than 99.5% and not more than the equivalent of 102% of the pure salt. Assayed by precipitation as magnesium ammonium phosphate with sodium phosphate in ammoniacal solution containing ammonium chloride, filtration after shaking and standing, washing with 2% ammonia solution and final ignition to pyrophosphate. The substance of the *U.S.P. XII* is required to contain

from 40 to 52% of water, and after ignition to the anhydrous salt contains not less than 99.5% of MgSO_4 .

Magnesii Sulphas Exsiccatus (B.P.C.). Contains from 62 to 70% of MgSO_4 , when precipitated as the magnesium ammonium phosphate compound and ignited to pyrophosphate. Magnesium sulfuricum siccatum, *P. Helv. V*, contains about two molecules of water and on heating to a low red heat loses from 22.5 to 25% of water.

Ammonii Persulphas (B.P.C.). $(\text{NH}_4)_2\text{S}_2\text{O}_8 = 228.2$. Contains not less than 98% of the pure substance. Assayed by the method of the *B.P.C.* 34: to 0.5 g. dissolved in 10 ml. of water, 50 ml. of N/10 oxalic acid and 0.2 g. of silver sulphate in 20 ml. of dilute sulphuric acid are added, after heating on a water-bath till evolution of carbon dioxide has ceased the mixture is diluted and the excess oxalic acid titrated with standard potassium permanganate.

Potassii Persulphas (B.P.C.). $\text{K}_2\text{S}_2\text{O}_8 = 270.3$. Assayed by the oxidising action of one equivalent of oxygen upon excess oxalic acid, as in the *B.P.C.* titration for Ammonii Persulphas, it contains not less than 98% of $\text{K}_2\text{S}_2\text{O}_8$.

Sodium, Ammonium and Potassium Persulphates are strong bleaching agents, the last known as Anthion, and the ammonium salts are used in photography to reduce dense negatives—they oxidise and then dissolve part of the silver. On adding barium chloride to a solution of a persulphate there is no precipitation but, on warming, barium sulphate is thrown down.

The ammonium salt is prepared by electrolysis of a solution of ammonium sulphate containing sulphuric acid. In presence of water it yields ozonized oxygen. It bleaches, and has been used as a hand disinfectant and to sterilise sponges.

Potassii Sulphas (B.P.C.). $\text{K}_2\text{SO}_4 = 174.3$. Contains not less than 99% of the pure salt, determined gravimetrically by precipitation and ignition of the barium compound. A 2% solution should not be acid to methyl orange. Potassii Sulfas, *N.F. VII*, contains 99%.

Sodii Sulphas (B.P.). $\text{Na}_2\text{SO}_4, 10\text{H}_2\text{O} = 322.2$. Assayed by weight of the ignited barium salt, it contains not less than 99% and not more than the equivalent of 102% of $\text{Na}_2\text{SO}_4, 10\text{H}_2\text{O}$. The substance of the *U.S.P. XII* contains not less than 99% of the anhydrous salt, after rendering anhydrous at 110° ; loss at 110° , 51 to 57%.

Sodii Sulphas Exsiccatus (B.P. Add. IV). $\text{Na}_2\text{SO}_4 = 142.1$. Loses not more than 5% of its weight at 100° , and then contains not less than 99% of the anhydrous substance. Assayed by precipitation with barium chloride and ignition of the insoluble sulphate. Complies with the tests for purity for Sodii Sulphas using 2/5 of the quantities. The *N.F. VII* substance loses not more than 3% at 100° , and is then of the same purity. Natrium sulfuricum siccatum, *P.G. VI*, contains not less than 88.6% of Na_2SO_4 .

Zinci Sulphas (B.P. Add. I). $\text{ZnSO}_4, 7\text{H}_2\text{O} = 287.5$. Assayed by the process of the British Pharmacopœia Add. I; a solution of

0.4 g. in 120 ml. of water, just acidified with dilute sulphuric acid and mercuric ammonium thiocyanate solution added, is set aside to crystallise; the filtered precipitate is washed with five 10 ml. quantities of diluted ammonium mercuric thiocyanate (1 : 50), and then titrated immediately after the addition of 5 ml. of water, 40 ml. of hydrochloric acid and 5 ml. of chloroform, with M/5 potassium iodate. It contains from 99.5% to the equivalent of 101% of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$. Zinci Sulfas, *U.S.P. XII*, contains from 55.58 to 61.0% of ZnSO_4 , corresponding to not less than 99% of crystalline substance and is assayed by precipitation as carbonate and ignition to oxide; limit of alkalis and alkali earths weighed as sulphate, 0.5%.

ACIDUM SULPHUROSUM

Acidum Sulphurosum (B.P.C.). Contains not less than 4.5% and not more than 5.5% *w/w* of SO_2 , corresponding to 5.76 to 7.05% *w/w* of H_2SO_3 . Sp. gr. about 1.025. Estimated by oxidation with excess standard iodine and titration with sodium thiosulphate.

Sulphurous acid is a strong reducing agent. For example, many colours are bleached by the sulphurous acid combining with the oxygen of any water present, hydrogen being liberated, which latter forms colourless compounds with the colours. These compounds may then be removed by washing.

The gas compressed in small cylinders was used for room disinfection, but formaldehyde (*q.v.*) is more frequently used now.

Official qualitative and quantitative methods for the determination of sulphurous acid in foods are described in *Methods of Analysis (A.O.A.C., 1940, 463)*.

Sulphur Dioxide in Industry.

Sulphur dioxide is one of the most commonly used gases. Apart from its occurrence in industry in the roasting of ores and the distillation of coal, it is used extensively in refrigeration, fumigation, and for bleaching. It is colourless, but possesses a distinctive odour, whilst some people taste it even more readily at the back of the mouth than they are able to smell it. At one time it was believed that sulphur dioxide in the air of towns did considerable damage, but much of the damage attributed to it may be caused by sulphuric acid, to which it is stated to be oxidised fairly readily. In the neighbourhood of blast furnaces vegetation may be attacked at a distance of several miles, conifers being particularly susceptible. In high concentration it acts as an asphyxiant, but, as generally encountered, it is an irritant. Concentrations of 400–500 parts per million are dangerous even for a short exposure. 20 parts per million will cause coughing in a short time, whilst not more than 10 parts per million can be inhaled for any considerable period. The least detectable odour is stated to be between 3 and 5 parts per million.

Detection and Determination. Three methods are in general use for the determination of sulphur dioxide in air, all of which have been adapted for the particular purpose in hand. For the

detection of comparatively large amounts of the gas, i.e., a concentration of 10 parts per million or more, the air is drawn through a test paper, prepared by soaking in a dilute solution of glycerin containing 1% of potassium iodate and 2% of potassium iodide, drying and using fresh. The colours obtained are compared with standard coloured charts. Sulphur dioxide in the air in amounts down to 1 part per 100,000,000 is determined by drawing a known volume of air, usually over a 24-hour period, through a 1% hyperol solution and determining the increase in acid formed either by titration with N/100 NaOH or by precipitation as barium sulphate. Active sulphur dioxide in the air is determined by exposing cylinders coated with a tragacanth suspension of lead peroxide and determining the quantity of lead sulphate formed.—(*Toxic Gases in Industry*, No. 3, D.S.I.R.)

Sodii Sulphis (B.P.C.). $\text{Na}_2\text{SO}_3 \cdot 7\text{H}_2\text{O} = 252.2$. Assayed by addition of excess standard iodine solution and titration with sodium thiosulphate, using starch mucilage indicator, a purity of not less than 94% should be indicated. Thiosulphate is limited by absence of turbidity on addition of hydrochloric acid to a 10% aqueous solution.

Sodii Metabisulphis (B.P. Add. IV). $\text{Na}_2\text{S}_2\text{O}_5 = 190.1$. By oxidation with excess of N/10 iodine in acid solution and back titration with N/10 sodium thiosulphate, it contains not less than 90% of $\text{Na}_2\text{S}_2\text{O}_5$. A test for absence of thiosulphate is included.

Sodii Thiosulphas (B.P. Add. I). $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O} = 248.2$. On titration with N/10 iodine, 99 to the equivalent of 101% of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ is indicated. Sodii Thiosulphas, U.S.P. XII, contains 99% of $\text{Na}_2\text{S}_2\text{O}_3$, determined on the anhydrous substance; loss at 40° to 50°, and afterwards at 100°, 32 to 37%. Natrium thiosulfuricum is official in the P.G. VI.

Decinormal sodium thiosulphate solution is stable for 5 months if prepared with 0.05% NaOH and 0.1% sodium benzoate as preservatives.—J. Ehrlich, *Industr. Engng Chem. (anal. Edn.)*, 1942, 5, 406.

Auri et Sodii Thiosulphas (B.P.C.). $\text{Na}_3\text{Au}(\text{S}_2\text{O}_3)_2 \cdot 2\text{H}_2\text{O} = 526.5$. Should retain its colour, physical properties and solubility when kept in closed phials at 75° for 24 hours. Assayed by the B.P.C. process for Au content by treatment of 0.8 g. in 50 ml. of water with 10 ml. of N/1 sodium hydroxide and 10 ml. of 20 vols. hydrogen peroxide, boiling off excess peroxide and acidifying with hydrochloric acid; the coagulated precipitate of gold is washed, dried, ignited and weighed. A gold content of 37.0 to 37.6% is required.

ACIDUM TANNICUM

Acidum Tannicum (B.P.). Obtained from the galls of various species of *Quercus*; it loses from 6 to 12% of its weight at 100°. Gums, dextrin, sugar and salts are limited by the addition of an equal volume of alcohol to a 20% aqueous solution, and no turbidity is shown on the addition of a half volume of ether. The

U.S.P. XII acid should lose not more than 12% of its weight at 100°.

The identification by chemical methods of drugs containing tannins.—A. H. Ware, *Pharm. J.*, ii/1925, 131.

A review of the tannins in astringent drugs.—A. H. Ware, *Pharm. J.*, ii/1926, 162.

Commercial examination of tannins by comparative methods.—W. B. Forbes, per *Yearb. Pharm.*, 1926, 293.

Garratt (*Drugs and Galenicals*) gives details for the application of the Lowenthal-Proctor method for determination of tannin in drug infusions.

Ammonium molybdate, 10% solution, gives a reddish-brown colour with tannic acid, also gallic acid, pyrogallol and tincture of catechu. May be used for colorimetric assay for tannin and drugs containing it.—J. Rae, *Pharm. J.*, i/1928, 539.

For a general account of the history, chemistry and distribution of the natural organic tannins see *The Natural Organic Tannins* by M. Nierenstein (J. & A. Churchill).

Albumini Tannas (U.S.P. XI). Loses not more than 6% at 100° for 3 hours; ash not more than 0.3%; water-soluble matter not more than 12%; residue insoluble in 1% sodium bicarbonate solution with pancreatin at 40° after drying at 110°, not more than 30%; residue insoluble in acid pepsin mixture at 40°, and after drying at 110°, not more than 40%.

Acetannin (B.P.C.). Loses not more than 3% of its weight at 100°. A limit of the colour produced on adding ferrous tartrate solution to water shaken with the substance is specified and is equivalent to a tannic acid limit of 0.2%. *Acidum Acetyltannicum, U.S.P. XI*, yielded not more than 15% of matter insoluble in 1% sodium bicarbonate solution and dried at 110°; water-soluble matter not more than 6%.

Acidum Gallicum (B.P.C.). $C_7H_6O_5 \cdot H_2O = 188.1$. Loss at 100°, not more than 10%. A 5% solution should be not more than faintly yellow and does not precipitate with gelatin or albumen solutions. The *N.F. VII* allows a 12% loss at 100° and 0.1% of ash.

Catechu (B.P.). The substance dried at 100° yields not more than 25% of water-insoluble matter. Loss at 100°, not more than 10%. Alcohol-insoluble matter dried at 100° and containing not more than occasional starch grains, not more than 30%. Ash, not more than 8%. Gambir, *N.F. VII*, yields not less than 70% of water-soluble extractive and not less than 60% of alcohol-soluble extractive; acid-insoluble ash limit, 0.5%.

Chloroform extractives not green.—Kay and Oldham, *Pharm. J.*, i/1938, 663.

Intensity of colour in chloroformic extract depends upon the particular sample rather than upon age.—R. E. Wagg, *Pharm. J.*, ii/1938, 27.

Catechu Nigrum (B.P.C.). Alcohol extractive, not less than 60%. Ash, not more than 8%.

Galla. The following tests for purity of Aleppo Galls are recommended by the Sub-Committee on Crude Drugs of the Committee on Pharmacy and Pharmacognosy:—Contains not more than 1% of foreign organic matter; ash, not more than 3%. Transfer 0.1 g. in No. 60 powder to a glass-stoppered vessel of about 300 ml. capacity. Add 100 ml. of boiling water, shake well, allow to stand for ten minutes, and cool quickly to about 20°. Add 50 ml. of N/10 iodine and 40 ml. of N/10 sodium carbonate. Shake gently, allow to stand for ten minutes, add 15 ml. of N/1 sulphuric acid and titrate the excess of iodine with N/10 sodium thiosulphate, using mucilage of starch as indicator; the iodine absorbed is equivalent to not less than 23 ml. of N/10 iodine.—(*British Pharmacopœia Commission Report*, No. 13, September 1939.)

Kino (B.P.C.). Extractive to boiling water, not less than 75%. Ash, not more than 2.5%. The *N.F. VII* requires Kino to yield not less than 60% of alcohol-soluble extractive and not less than 75% of water-soluble extractive.

Tests for Identity and Purity. The following tests for East Indian, Malabar, Madras or Cochin Kino are recommended by the Sub-Committee on Crude Drugs of the Committee on Pharmacy and Pharmacognosy:—To an aqueous solution add test-solution of ferric chloride; a dark green precipitate is produced. To an aqueous solution add solution of sodium hydroxide; a reddish-violet colour is produced. To 5 ml. of a filtered 1% solution in cold water, add 2 ml. of *N/10* iodine; a slight precipitate is produced; add 0.5 ml. of dilute solution of ammonia; the precipitate dissolves (distinction from butea kino). To 5 ml. of a filtered 1% solution in cold water, add 2 ml. of *N/10* iodine; a slight precipitate is produced; boil for one minute, cool, and add 0.5 ml. of dilute solution of ammonia; the precipitate is insoluble (distinction from eucalyptus kino). **Tests for Purity.**—Ash, not more than 2.5%. Yields not less than 75% of extractive soluble in hot water, as determined by the following method:—stir 1 g. in powder with 50 ml. of boiling water, and decant the liquid through a filter into a 200-ml. flask; stir the residue with two further quantities of 50 ml. of boiling water, decanting the liquids through the filter; transfer the insoluble residue to the filter and wash it with hot water; cool the filtrate and washings and make up to 200 ml. with water; evaporate 50 ml. to dryness in a flat-bottomed shallow dish and dry at 100°.—(*British Pharmacopæia Commission Report*, No. 13, September 1939).

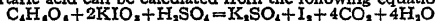
Kino Eucalypti (B.P.C.). Alcohol (90%) extractive, not less than 80%. Ash, not more than 3%.

ACIDUM TARTARICUM

Acidum Tartaricum (B.P.). $C_4H_6O_6 = 150.0$. Loses not more than 1% at 100° and then contains 99.5% of the pure substance. Acidum Tartaricum, *U.S.P. XII*, contains 99.7% of $H_2C_4H_4O_6$, when dried over sulphuric acid for 5 hours.

Detection in Syrups and Lemonades. Dilute 100 ml. of the liquid with 20 ml. of water and filter into a 300 ml. test-glass. Add 20 ml. of strong solution of calcium acetate, made by dissolving $CaCO_3$, 32 g. in glacial acetic acid 120 ml. and diluting to 1 litre with distilled water. Mix, stir well, and set aside for 72 hours. In presence of tartaric acid an evident crystalline deposit will be seen which is identified by micro-examination. Confirm by applying Denigé's Test (resorcin and sulphuric acid).—per *Yearb. Pharm.*, 1926, 179.

Determination of Tartaric Acid. About 0.3 g. of the acid is added to a flask containing 1 g. of potassium iodate, a few drops of water and 80 ml. of concentrated sulphuric acid, and the mixture is heated for about 30 minutes until most of the iodine is expelled. Water is added and the last traces of iodine are removed by boiling. By estimation of the remaining potassium iodate (by adding potassium iodide to an aliquot portion and titrating with thiosulphate) the amount of tartaric acid can be calculated from the following equation:—



—*J. chem. Soc. Abstr.*, ii/1924, 73.

Determination as Lead Tartrate. Lead tartrate is quantitatively precipitated from solutions containing tartrates, neutralised to phenolphthalein, by means of 5% lead nitrate solution, of which about 15 ml. is required for 2 g. of acid. After standing 2 hours the precipitate is collected on a Gooch crucible, washed, dried at 105°, and weighed. 1 g. of lead tartrate corresponds to 0.423 g. of $C_4H_6O_6$.—C. H. Manley, *Analyst*, 1937, 526.

Garratt (*Drugs and Galenicals*) recommends the method of King (*Analyst*, 1933, 135) for the determination of tartaric acid in pharmaceutical preparations, in which calcium racemate is separated in dilute acetic and hydrochloric acids.

An official quantitative method for the determination of tartaric acid in baking powders is described in *Methods of Analysis (A.O.A.C., 1940, 188)*.

Potassii Tartras (*B.P.C.*). $C_8H_8O_{12}K_4 \cdot H_2O = 470.5$. Contains not less than 99% of $C_8H_8O_{12}K_4 \cdot H_2O$. Assayed by titration of the alkaline residue, on gentle ignition, with standard sulphuric acid to methyl orange.

Potassii Tartras Acidus (*B.P.*). $C_4H_5O_6K = 188.1$. The substance dried at 100° contains not less than 99.5% of the pure compound. Loss at 100° , not more than 1%. Assayed by titration of the boiling solution with N/5 sodium hydroxide to phenolphthalein. Potassii Bitartras, *U.S.P. XII*, contains 99.5% of $KHC_4H_4O_6$ after drying at 100° . The *Fr. Cx.* 1937 substance contains 99% of the pure salt and loses not more than 1% at 100° .

Cream of Tartar Substitutes. These are usually calcium acid phosphate. A sample examined by Evans was a mixture of cream of tartar with sodium acid sulphate, but in such proportion that the whole of the tartaric acid would be liberated with the cream of tartar in solution, with an excess of about 10% $NaHSO_4$ still remaining. Another sample was a mixture of "dry" calcium acid phosphate and sodium chloride.

Sodium acid pyrophosphate, $Na_2H_2P_2O_7$, is also used as the acidic constituent of baking powders in place of tartaric acid and cream of tartar.

Sodii et Potassii Tartras (*B.P.*). $C_4H_4O_6NaK \cdot 4H_2O = 282.2$. By titration with standard sulphuric acid of the carbonised alkaline residue, using methyl orange as indicator, a purity of not less than 99% and not more than the equivalent of 104% should be found. Potassii et Sodii Tartras, *U.S.P. XII*, loses 21 to 26% of water at 150° , and then contains not less than 99% of the pure anhydrous substance. A test for limit of ammonium compounds is included.

ACONITUM

Aconitum (*B.P.*). The dried root of *Aconitum Napellus*; should contain not more than 2% of other organic matter. The Pharmacopœia states that there is no trustworthy chemical method of assaying the root, and does not consider the drug sufficiently important medicinally to include a biological method. Aconitum, *N.F. VII*, contains not more than 5% of its stems and not more than 2% of other foreign organic matter. 0.1 g. possesses an activity equivalent to not less than 0.150 mg. of reference aconitine when extracted by the method for Tinctura Aconiti, *N.F. VII*, and administered subcutaneously to guinea-pigs. Pulvis Aconiti I.A. is adjusted, by dilution with rice starch if necessary, to contain 0.5% of total alkaloids.

Aconiti Radix of the 1914 Pharmacopœia was assayed for ether-soluble alkaloids of which it was required to contain not less than 0.40%. A tincture was prepared from 10 g. by maceration and percolation with 75% alcohol, and was then evaporated to dryness at a temperature not above 60° ; a filtered solution of the residue dissolved in 5 ml. of N/10 sulphuric acid and 20 ml. of water and washings was made ammoniacal with 2 ml. of solution of ammonia and shaken for one minute with 25 ml. of ether; after evaporation the aqueous liquid was extracted with three subsequent portions of 20 ml. of ether; the ethereal solutions, filtered and

evaporated to dryness, were dried at 60° and the residue titrated with N/20 sulphuric acid, using cochineal as indicator. Tuber Aconiti, *P. Helv. V*, consists of the roots of *Aconitum Napellus* dried rapidly at about 40° and then for one hour at 50° and containing not less than 0.8% of alkaloid. The powder for administration is adjusted with lactose to contain 0.5% of alkaloid.

Assay. The method is based on a determination of the benzoic acid produced by the hydrolysis of the alkaloids, and therefore determines aconitine plus benzoylaconine but not aconine. The total alkaloids are first determined, after extraction in the usual way, by the method of indirect titration, and the solution is evaporated practically to dryness in a current of air on the water-bath. 10 ml. of N/2 alcoholic potash is added, and the mixture refluxed for two hours, and evaporated to dryness. The residue is dissolved in water, acidified with sulphuric acid, and shaken out into a mixture of 80 ml. of light petroleum and 20 ml. of ether for some hours. 80 ml. of the ethereal layer is then evaporated in a current of air, the residue dried *in vacuo* to remove acetic acid, and then titrated with N/100 sodium hydroxide, using a micro-burette.—W. A. van Bronkhorst, *Pharm. Weekbl.*, 1935, 1056.

The Poisons Sub-Committee of the Society of Public Analysts have found that more consistent results were obtainable when assays were based on total alkaloidal content rather than upon the ether-soluble portion. By comparison with biological methods, it was concluded that the chemical assay for total alkaloids generally gives good indication of toxicity, but it should not be assumed that the chemical determination of total alkaloids will give more than an approximate measure of the therapeutic efficacy of the drug. **Recommended Method:**—Shake 10 g. of the drug in No. 60 powder in a suitable stoppered percolator with 100 ml. of ether-chloroform mixture (3 : 1) and allow to stand 15 minutes. Add 5 ml. of dilute solution of ammonia and shake for 1 minute at 10-minute intervals during 1 hour. Allow the liquid to percolate into a separating funnel. When the liquid ceases to flow, pack the drug firmly and continue percolation with mixed solvent until complete extraction of the alkaloids is effected. (Test for complete extraction by evaporating 2 ml. of percolate, dissolving the residue in a few drops of 0.1 N sulphuric acid and adding a few drops of 0.1 N iodine; no precipitate or turbidity should be formed.) To the percolate add 30 ml. of N sulphuric acid, or sufficient to render it faintly acid. Shake, allow to separate and transfer the lower layer to another separating funnel A. Complete the extraction of the alkaloids from the organic solvent, using 10 ml. portions of 0.1 N sulphuric acid and testing for complete extraction by the iodine test. Wash the mixed solutions in A with about 10 ml. of chloroform, and run off the latter to a second separating funnel B, containing 20 ml. of 0.1 N sulphuric acid. Shake, allow to separate and reject the chloroform. Wash the liquid in funnel A with two further 5 ml. portions of chloroform, transferring each in turn to funnel B, washing, and rejecting the chloroform. Transfer the acid liquid from funnel B to funnel A, make just alkaline with dilute solution of ammonia and add 2 ml. excess. Shake with successive portions of chloroform until complete extraction is effected, washing each chloroform extract with the same 20 ml. of water. Distil off the chloroform, add 2 ml. of absolute alcohol to the residue and evaporate at not more than 60°. Dry at a temperature below 60° for 30 minutes. Dissolve the residue in 2 ml. of neutral 95% alcohol, warm to dissolve, add 20 ml. of 0.02 N sulphuric acid and 10 ml. of water, cool and titrate with 0.02 N sodium hydroxide, using methyl red indicator. 1 ml. of 0.02 N acid = 0.01291 g. of alkaloids calculated as aconitine.—*Analyst*, 1942, 289.

Determination of Aconitine in Preparations. Aconitine cannot be separated from benzoylaconine by any usual method for isolating alkaloids, and the following assay depends on the extraction of the two compounds with ether from a buffered solution and calculation of the amount of aconitine present from a study of their dissociation constants. To 10 ml. of the liquid extract or 100 ml. of the tincture add 1 ml. of 10% sulphuric acid and evaporate the alcohol on a steam-bath. Add about 20 ml. of water, filter off the insoluble residue, washing the filter paper with small quantities of water and finally with one or two portions of acidified water. Shake out with ether, rejecting the ether. Add 10% ammonium hydroxide until slightly alkaline to litmus and completely extract the alkaloids with ether. To the combined ethereal extracts add 10 ml.

of N/100 acid, evaporate the ether, and titrate with N/100 alkali using methyl red as indicator. Each ml. of N/100 acid is equivalent to 6.45 mg. of total alkaloids calculated as aconitine. Transfer the neutral solution to a separator containing 50 ml. of the buffer solution (0.0159 N ammonium hydroxide and 0.75 N ammonium chloride) and extract with four successive portions of ether, each of 25 ml., shaking each time for about 10 minutes. Again add 10 ml. of standard acid to the combined ethereal extracts, evaporate the ether and titrate the excess acid as before. The weight in mg. of aconitine, A, is given by the equation $A = 7.236y - 0.1106h$, where y = ml. of standard acid used, and h = total alkaloids.—G. Baker and C. B. Jordan, *J. Amer. pharm. Ass.*, 1936, 291.

Farr and Wright found in aconite extract an average of 0.43% total alkaloid. The amount in the root is about twice that in the leaf. They found in dry root extract from 1.2 to 6%, English root being the best. A method of making the extract is outlined. The average yield of the dry extract was 25.9%, the ether-soluble alkaloid in this averaging 1.95%. Foreign root yielded 30% with an average of only 0.68% ether-soluble alkaloid. A standard of 1% proposed. The dose of this extract would be $\frac{1}{2}$ to $\frac{1}{4}$ grain. Foreign root is very mixed owing to mode of collection.—*Pharm. J.*, i/1913, 216.

Fluidextractum Aconiti (N.F. VII). 1 ml. has a potency equivalent to from 1.4 mg. to 1.6 mg. of reference aconitine, when assayed by the biological method used for tincture of aconite.

Tinctura Aconiti (B.P.C.). An unstandardised tincture prepared by percolation of 150 g. of powdered root with 70% alcohol to 1 litre. Alcohol content, 67 to 69% *v/v* of ethyl alcohol. The tincture of the B.P. '14 was standardised to contain 0.04% of ether-soluble alkaloids. Tinctura Aconiti I.A., prepared with 70% alcohol, contains 0.05% of total alkaloids.

Assay of Aconite Tincture and Liniment. Loss of time caused by filtering the acid liquid can be minimised thus:—Evaporate 15 ml. of liniment or 100 ml. of tincture at low temperature to remove bulk of the alcohol. Add 5 ml. of 10% sulphuric acid. Shake with 20 ml. of petroleum ether, rinse same with water and extract with ether after making alkaline. Evaporate the ether extracts and titrate.—E. J. Chappel and N. L. Allport, *Yearb. Pharm.*, 1920, 444.

Biological Methods of Assay.

Tinctura Aconiti (N.F. VII), when assayed biologically on guinea-pigs, is required to possess a potency in 1 ml. equivalent to not less than 0.140 mg. and not more than 0.160 mg. of reference aconitine. In the assay, the guinea-pigs must all be from one colony and be kept under identical conditions. The doses are determined of reference aconitine and of the sample being tested, which will kill not more than seven and not less than three of groups of ten animals, within 6 hours. The doses are considered equivalent if the mortalities in each case differ by not more than two animals. The accuracy of this standardisation is stated to be $\pm 20\%$.

Toxicity Test on Mice. Dyer (*Quart. J. Pharm.*, 1930, 626) has suggested the determination of the toxicity by injection of a tincture into mice to find the dose which kills fifteen out of thirty mice. The toxicity, however, cannot be expressed in terms of this dose since when one sample is tested in different laboratories the dose differs (see Broom, Burn, Gaddum, Trevan and Underhill, *Quart. J. Pharm.*, 1932, 33). Each sample must, therefore, be compared for toxicity with a standard, which can be prepared by mixing a series of ten samples of the dried and powdered root of *Aconitum Napellus*. This standard would have to be distributed from some central institution. By comparing a tincture prepared from the sample with that prepared from the standard and finding the dose of each which kills fifteen out of thirty mice, then if the unknown sample is more toxic than the standard, it can be adjusted by dilution so as to be equal to the standard.

Aconitina (B.P.C.). $C_{34}H_{47}O_{11}N = 645.4$. M.p. when heated rapidly, 196° to 200° , acetic acid being evolved. The amount of pseudaconitine present is limited: 10 mg. dissolved in and evaporated with 5 drops of fuming nitric acid produces a yellow residue which should show no red colour on moistening

with N/2 alcoholic potassium hydroxide. Aconitina, *U.S.P. X*, dissolved in water with a little acetic acid and injected under the skin of the abdomen of guinea-pigs, had a minimum lethal dose of from 0.000000055 g. to 0.000000065 g. for each gramme of body weight of guinea-pig, two-thirds of the number of guinea-pigs being tested dying within 6 hours. Aconitinum, *P. Helv. V*, contains not less than 99.1% of $C_{34}H_{47}O_{11}N$ and is determined by titrating a solution in a known excess of N/10 hydrochloric acid with N/10 sodium hydroxide using methyl orange.

Aconitine gives a reddish-violet colour when warmed with resorcinol and sulphuric acid, and the liquid becomes colourless with a blue fluorescence when made alkaline with strong sodium carbonate solution; a residue of total alkaloids of aconite gives the same reddish-violet colour, but when made alkaline a deep purple colour is produced with a green fluorescence.—C. Bruegas, *Ann. Falsif.*, 1932, 25, 147.

Pseudaconitine. A crystalline alkaloid obtained from Indian (or Nepaul) aconite, *A. laciniatum*, melts at 201° and has the constitution of acetyl-veratroyl-pseudaconine.

Most of the Japanese aconite plants contain several isomers and closely related aconitines. Methods of extraction and isolation of 6 isomers.—*Yearb. Pharm.*, 1925, 48, 49.

ACRIFLAVINA

Acriflavina (*B.P. Add. I*). A 2% solution in water at 30°, and also a 0.2% solution in normal saline at 30°, should remain clear and free from sediment on standing in the dark for 24 hours at 15° to 20°. Residue on ignition with sulphuric acid, not more than 1%. Acriflavina, *N.F. VII*, a mixture of 2 : 8-diamino-10-methyl-acridinium chloride and 2 : 8-diamino-acridine and similar to euflavine, after drying to constant weight at 100°, contains 13.3 to 15.8% of Cl. Loss at 100°, not more than 7%. Residue on ignition with sulphuric acid, not more than 3.5%; matter insoluble in warm water, not more than 0.5% when dried at 100°. Assayed by digestion with potassium permanganate, sulphuric acid and silver nitrate solution, decolorisation with hydrogen peroxide and subsequent gravimetric estimation of the precipitated silver chloride. Acriflavinae Hydrochloridum, *N.F. VII*, loses not more than 7% over sulphuric acid, and then contains 23 to 24.5% of Cl; residue on ignition with sulphuric acid, not more than 1%.

Assay. The following process for the total diaminoacridines in acriflavine is recommended by the Sub-Committee on Synthetic Chemicals of the Committee on Pharmaceutical Chemistry:—Dissolve about 2 g., accurately weighed, in 250 ml. of water, and dilute with water to 750 ml. Adjust the reaction of the solution, at room temperature, by the addition of N/1 hydrochloric acid, until faintly acid to Congo-red paper, and then add 5 g. of sodium acetate. Add 50 ml. of M/10 potassium ferricyanide, stirring during the addition, allow to stand for ten minutes, filter through a Buchner funnel, and wash the precipitate with three successive quantities of 50 ml. of water. Mix the filtrate and washings, and add separately 10 ml. of hydrochloric acid, 10 g. of sodium chloride, 1 g. of potassium iodide, and 3 g. zinc sulphate, dissolved in 10 ml. of water, mixing after each addition. Allow to stand for three minutes, and titrate the liberated iodine with N/10 sodium thiosulphate, using mucilage of starch as indicator. When the titration is nearly complete, allow to stand for a further three minutes, and then complete the titration. Determine by :

control experiment the number of ml. of N/10 sodium thiosulphate equivalent to 50 ml. of the M/10 potassium ferricyanide. Calculate the volume of M/10 potassium ferricyanide required by the acriflavine; each ml. of M/10 potassium ferricyanide is equivalent to 0.08883 g. of $C_{14}H_{14}N_2Cl.HCl$.—(*British Pharmacopæia Commission Report*, No. 12, May 1939).

Commercial acriflavine consists of a mixture of approximately equal parts of the hydrochloride of 2:8-diaminomethylacridinium chloride and diaminoacridine dihydrochloride. The solubility of acriflavine depends on the proportions in which the two compounds are present. The methylacridinium compound has a solubility of 0.4%, and the diaminoacridine dihydrochloride of 0.3%. A synthetic mixture of equal parts has a solubility of 1%. The difference between this figure and that for acriflavine is due to the existence of diaminoacridine hydrochloride in an unstable but more soluble form. Mixtures of approximately equal parts of the two substances obtained by adding hydrochloric acid to a warm concentrated solution containing them have a solubility in agreement with the B.P. figure of 1 in 3.—*M. Gailliot, Quart. J. Pharm.*, 1934, 63.

Solubility. The solubility increases with increasing proportions of diaminoacridine dihydrochloride up to 50%. On standing, most solutions precipitate owing to hydrolysis. An acidity corresponding to more than about 1% of HCl results in a lowering of the solubility.—*G. F. Hall and A. D. Powell, Quart. J. Pharm.*, 1936, 510.

From commercial samples of acriflavine containing 30 to 40% of diaminoacridine, a product containing only about 12% of this material may be obtained by treatment with caustic soda. Recrystallisation of the partly purified product from water then suffices to remove the whole of the remaining diaminoacridine.—*J. Marshall, Quart. J. Pharm.*, 1934, 514.

Results of Analysis of some Commercial Flavines

| Samples of Acriflavine | "Total flavines" (ferricyanide) as acriflavine per cent. | Acriflavine from chloride (uncorr.) per cent. | Diamino- acridine dihydrochlor. (alkali pptn.) per cent. |
|---------------------------|--|--|--|
| A | 97.2 | 96.5 | 35.2 |
| B (1) | 96.1 | 96.8 | 11.0 |
| B (2) | 98.6 | 96.7 | 20.0 |
| C | 93.6 | 94.6 | 42.0 |
| Samples of Euflavine | "Total flavines" (ferricyanide) as euflavine per cent. | Euflavine from chloride corrected for NaCl per cent. | Diamino- acridine monohydro- chlor. (alkali pptn.) per cent. |
| A | 91.4 | 94.2 | 33.5 |
| B (1) | 93.9 | 97.2 | — |
| B (2) | — | — | 5.5 |
| C | 87.5 | 95.6 | 25.5 |
| Samples of Proflavine | "Total flavines" (ferricyanide) as proflavine (sulphate) per cent. | Proflavine (sulphate) from sulphate per cent. | Proflavine (sulphate) (alkali pptn.) per cent. |
| A | 92.4 | 89.7 | 92.1 |
| B | 97.7 | 94.4 | — |

—*G. F. Hall and A. D. Powell, Quart. J. Pharm.*, 1934, 522.

Determination of Flavine Compounds. For determining flavine compounds in preparations the ferricyanide method must be modified as follows:—

General Method. The solution derived from preliminary treatment, and containing from 0.02 to 0.2 g. of the diaminoacridine derivative, is diluted to approximately 200 ml. and rendered faintly acid to Congo-red paper. 1 g. of sodium acetate crystals is added, followed by sufficient excess of M/50 potassium ferricyanide with stirring during the addition (10 to 30 ml. according to the amount required for precipitation). The mixture is allowed to stand for 30 minutes and then filtered through a Buchner funnel. The precipitate is washed with three successive quantities of 10 ml. of water, and to the combined filtrate and washings 5 ml. of hydrochloric acid (sp. gr. 1.16), 1 g. of sodium chloride, 0.5 g. of potassium iodide and 5 ml. of 30% zinc sulphate solution are added, with mixing after each addition. After standing for 3 minutes, the liberated iodine is titrated with N/100 sodium thiosulphate. A blank determination is also made, using the same amount of M/50 ferricyanide reagent. 1 ml. of M/100 ferricyanide precipitated is equivalent to 0.00888 g. of acriflavine, 0.00779 g. of euflavine, or 0.00921 g. of proflavine.

Dressings. Extract 20 g. or other convenient quantity, in a Soxhlet extractor, with alcohol (95%) slightly acidified with hydrochloric acid (250 ml. of alcohol and 2 ml. of dilute hydrochloric acid). Usually 3 hours are required. Transfer the extract to a beaker, add 50 ml. of water, evaporate to remove the bulk of the alcohol, and add, whilst still hot, 25 ml. of chloroform. Mix thoroughly, cool, transfer to a separator, allow to separate and then complete the removal of fats by extraction with chloroform (25, 25 ml.). Wash these combined extracts with two successive quantities of 10 ml. of water, made slightly acid with one or two drops of dilute hydrochloric acid. If emulsions are formed, break with a little alcohol. Evaporate the united aqueous solutions to low volume to remove alcohol, dilute to about 200 ml., add dilute sodium hydroxide solution until slightly acid to Congo-red paper, and complete as the general method.

Pessaries and Emulsions Containing Fat or Oil. A suitable quantity of the preparation (generally 25 to 50 g.) is treated with about 50 ml. of chloroform to dissolve the basis. The solution is then transferred to a separator and extracted with 20 ml. of water containing 2 ml. of dilute hydrochloric acid, followed by two extractions with 10 ml. of water containing 0.5 ml. of dilute hydrochloric acid. The combined acid-aqueous extracts are washed with 25 ml. of chloroform: any medicament taken into the chloroform is re-extracted with 10 ml. of water containing 1 or 2 drops of dilute hydrochloric acid. If emulsions form, they can be broken by the addition of alcohol. The combined acid-aqueous extracts are transferred to a beaker, evaporated sufficiently to remove alcohol if present, cooled, and diluted to about 200 ml. Sodium hydroxide is added until the liquid is only slightly acid to Congo-red paper, and the estimation completed by the general method.

The method can also be applied to preparations containing glycerin and gelatin but errors of 5 to 10% arise owing to the presence of reducing substances. —G. F. Hall and A. D. Powell, *Quart. J. Pharm.*, 1937, 486.

Methylene Blue Determination of Acridines. Acridines and their salts are precipitated by picric acid. By adding an excess of picric acid, removing the precipitate, and estimating the amount of acid unused by titration with methylene blue, the acridine may be determined. The reagents required are (1) N/100 picric acid, prepared by dissolving 2.29 g. of picric acid in water and diluting to 1 litre, and (2) N/1000 methylene blue, prepared by dissolving 3.8 g. of methylene blue in water containing about 3 ml. of chloroform and diluting to 1 litre to produce an approximately N/100 solution; N/1000 methylene blue is prepared by dilution and standardisation against N/1000 picric acid, which is made by dilution of the above N/100 solution. **Methods.**—(a) 2:8-Diaminoacridine (base). Dissolve about 0.1 g. in 30 ml. of 0.5% acetic acid in a 200 ml. graduated flask. Add a measured excess of N/100 picric acid (0.1 g. requires theoretically 47.85 ml.) and adjust the volume to 200 ml. with water. Mix well and keep for at least an hour in a refrigerator. An aliquot portion (20 ml.) of the filtrate is transferred to a separating funnel containing chloroform and calcium carbonate, and N/1000 methylene blue run in from a burette. The methylene blue picrate formed is extracted by the chloroform, on shaking, leaving the aqueous layer yellow. On continuing the titration, with frequent changes of chloroform, the yellow colour gradually fades and the end-point is taken when one drop of methylene blue solution gives a faint blue colour, which is not removed by the chloroform, to the almost

colourless, aqueous layer. (b) *Proflavine*. 0.1 to 0.2 g. of sample (0.1 g. theoretically requires 32.56 ml. of N/100 picric acid) is used, and it is advisable to keep the reaction mixture in a refrigerator for at least four hours, otherwise the procedure is the same as for the base. Details are also given for combined determinations of base and sulphate. (c) 2 : 8-Diamino-10-methylacridinium chloride. Determine as for proflavine, using 0.1 to 0.2 g. of sample (0.1 g. theoretically requires 38.46 ml. of N/100 picric acid). In this case the picrate precipitated is orange and amorphous, contrasted with the yellow, crystalline appearance of 2 : 8-diaminoacridine picrate. Chloride may be determined by applying Volhard's method to a portion of the filtrate, providing only a small excess of picric acid has been used, and the precipitated silver chloride and thiocyanate are filtered off before the end-point is reached. (d) *Acriflavine* (and similar mixtures of acridine hydrochlorides). The total "flavine" content can be determined by adding picric acid to a solution and titrating the excess of picric acid. The result is preferably expressed as percentage of diamino-methylacridinium chloride (euflavine). The chloride content may be determined by Volhard's method. An approximate separation of the two constituents may be effected by the silver oxide method of Gailliot (*Quart. J. Pharm.*, 1934, 63). Approximately 0.5 g. of sample is dissolved in 30 ml. of water and added to freshly prepared silver oxide. The mixture is stirred for five minutes, filtered through sintered glass, and the precipitate washed with 5 ml. of water, which is added to the filtrate. The filtrate contains diamino-methylacridinium hydroxide. After slight acidification with hydrochloric acid it is treated with a known excess of N/100 picric acid (120 ml.), and the solution made up to volume. After standing for four hours the picrate is filtered off and the excess picric acid determined. The precipitate containing the diaminoacridine is removed from the filter and treated with 50 ml. of 4% acetic acid. To the mixture is added 80 ml. of N/100 picric acid and the volume of solution adjusted to 250 ml.; after filtration the excess of picric acid is determined.—A. Bolliger, *Quart. J. Pharm.*, 1940, 1.

Euflavina (*B.P.C.*). Should contain not less than 93% of the pure substance, calculated with reference to the sample dried at 120° at which temperature it should lose not more than 7% of its weight. A 0.2% solution should remain clear when kept in the dark for 24 hours. Assayed by the recommended *B.P.* process for acriflavine (*see* p. 49). 1 ml. of M/10 potassium ferricyanide is equivalent to 0.07788 g. $C_{14}H_{14}N_3Cl$. Chlorhydrate de Diamino-Acridine is official in the *Fr. Cx.* 1937.

Linteum Euflavinæ (*B.P.C. Supp. I*). Contains not less than 0.075% of euflavine, $C_{14}H_{14}N_3Cl$. Assayed by the method described for dressings on p. 51.

Proflavinæ Sulphas (*B.P. Add. IV*). $C_{13}H_{11}N_3 \cdot H_2SO_4 = 307.19$. Loses at 100°, not more than 10% and then contains not less than 98% of the pure salt. 1 g. in 250 ml. of water at 35°, and 0.2 g. in 100 ml. of water at 50° and at 20° with 0.9 g. of sodium chloride, remain clear and free from sediment when kept in the dark for 24 hours at 15° to 20°. Residue on re-ignition with sulphuric acid, not more than 1%. Assayed by precipitation with M/10 potassium ferricyanide as recommended for the *B.P.* assay of acriflavine on p. 49.

ADEPS LANÆ

Adeps Lanæ (*B.P. Add. I*). M.p., 34° to 40°. Acid value not more than 1. Saponification value (2 hours boiling), 94 to 106. Iodine value, to 32. Loss at 100°, not more than 0.5%. Ash not more than 0.15%. Complies with the revised limit test for

chlorides described under Adeps. The *U.S.P. XII* substance melts between 38° and 42°; loss on a water-bath, not more than 0.5%; ash, not more than 0.1%; acids in 10 g. equivalent to not more than 2.0 ml. of N/10 sodium hydroxide; iodine value, 18 to 36.

Alcoholia Lanæ (*B.P. Add. VI*). Assayed by precipitation with digitonin solution at 60°, collecting the residue on a Gooch crucible and drying at 100°, it contains not less than 28% of cholesterol. M.p. not below 54°; acid value not more than 3; saponification value not more than 12; acetyl value 130 to 140. Loses not more than 0.5% when heated at 100° for 1 hour; ash not more than 0.3%. An 8% solution in neutral alcohol (90%) is not alkaline to phenolphthalein.

Adeps (*B.P. Add. I*). After the preliminary treatment described in the *B.P.*, by melting 10 to 20 g., cooling until a turbidity appears, stirring until homogeneous, and setting aside for five hours with the container in water at 10° to 12°, or in running water for twenty-four hours, it should melt between 34° and 41°. Refractive index at 60°, 1.452 to 1.455. Acid value, not more than 1.2. Saponification value, 192 to 198. Unsaponifiable matter, not more than 0.5%. Iodine value, 52 to 66. The melting-point of the lard stearin obtained by the matter insoluble in ether at 16° to 20° should be not lower than 63° and is usually higher than 63.4°; a low melting-point indicates the presence of beef stearin. Sesame oil is tested for by the general pharmacopœial test with hydrochloric acid containing 1% of sucrose, giving no pink colour on shaking and setting aside for 5 minutes; and cottonseed oil by the general *B.P.* test of mixing with an equal volume of a mixture of equal parts of amyl alcohol and 1% solution of precipitated sulphur in carbon disulphide and immersing the container in boiling water for 30 minutes, when no red colour develops. A limit of chlorides in which the turbidity with silver nitrate produced by the filtrate from 1 g. boiled with alcohol, diluted and acidified, is not greater after 5 mins. standing than that produced by 0.5 ml. of N/50 hydrochloric acid similarly treated, is included. Adeps, *U.S.P. XII*, melts at 36° to 42°, after melting just sufficiently to draw into a capillary, cooling at 10° for 24 hours or at 0° for 2 hours, and the point taken when melted just sufficiently to rise in the capillary when heated in a water-bath at about 0.5° rise per minute. It is tested for cottonseed fats by warming 5 ml. with an equal volume of alcohol containing 0.05 g. of silver nitrate and one drop of nitric acid on a water-bath for 5 minutes, when no red or brown colour nor brown line of contact develops. The microscopical appearance of the separated stearin, which should appear as flat, rhomboidal plates with one oblique end and irregularly placed, and not as cylindrical sharp-ended rods or needles in fan-shaped clusters, excludes beef fat. Saponification value, 195 to 203; iodine value, 46 to 70; solidification point of the fatty acids, 36° to 42°. The *Fr. Cx.* 1937 describes a test for cottonseed oil (limit 5%) in lard,

when the fat and a silver nitrate solution in alcohol with nitric acid, kept in the water-bath for 10 minutes, does not become red or brown.

The Food and Drug Administration of the U.S. Dept. of Agriculture defined lard as the rendered fresh fat from hogs, in good health at the time of slaughter, free from rancidity and containing not more than 1% of substances other than fatty acids and fats. *Leaf lard*: lard rendered at a moderately high temperature from the internal fat of the abdomen of the hog, excluding that adherent to the intestines, and with iodine number not greater than 60. *Neutral lard*: lard rendered at low temperatures.—*S.R.A., F.D. No. 2, Rev. 5, Nov. 1936.*

Lard is very fully standardised because it is very easily adulterated and if any standards at all are given they should be as many as will give the greatest assurance that genuine lard is being used. The directions for the treatment of the sample before determining the melting-point are complicated but do not go far enough because the method adopted for the preliminary treatment may seriously affect the result. The melting-point range does not include all genuine lards.—*N. Evers, Pharm. J., i/1933, 196.*

A method for the approximate determination of some of the unsaturated minor component acids of pig and other fats is by separation of the fatty acids as lithium salts from acetone. The yield of ether-insoluble bromo-derivative products is determined and from this the percentage of C_{20-22} acids is obtained. The iodine and thiocyanogen values of the acids are determined and from the results the total saturated oleic and linoleic acids are found.—*T. P. Hilditch and W. H. Pedelty, Analyst, 1939, 640-647.*

Extraordinary variations occur in lard according to the age of the pig and the character of the food. Iodine value, refractive index, Bömer value, microscopical appearance of crystals, are all examined for various lards and mixtures.—*R. W. Sutton et al., Analyst, 1940, 623-636.*

Sevum (B.P.). M.p., 45° to 50° (by introducing into a capillary 0.75 to 1 mm. diameter, and, if by melting, cooling at 0° for 2 hours or standing for 24 hours and taking the temperature at which the substance is completely transparent). Refractive index at 60° , 1.449 to 1.451. Acid value, not more than 2.0. Saponification value, 192 to 195. Iodine value, 33 to 46. Sevum Præparatum, *U.S.P. XII*, melts at 45° to 50° ; congealing point, 37° to 40° ; saponification value, 193 to 200; iodine value, 33 to 48; the acid value of 10 g. is equivalent to not more than 6 ml. of N/10 NaOH.

ADRENALINA

Adrenalina (B.P.). $C_9H_{13}O_3N=183.1$. M.p., 205° to 212° with decomposition (the rate of rise of temperature being 10° per minute); specific rotation of a 4% w/v solution in normal hydrochloric acid, -50° to -53° . Epinephrina, *U.S.P. XII*, has an $\alpha_{D_{25}}$, determined on a 5% w/v solution of the substance, dried over sulphuric acid, in N/2 hydrochloric acid, of -50° to -53.5° . Liquor Epinephrinæ Hydrochloridi, *U.S.P. XII*, has a potency equivalent to a 0.1% solution of Reference Standard Epinephrine. Assayed biologically by measuring the rise in the systolic blood pressure of the dog and comparing it with that obtained with a standard solution.

Suprarenalum (N.F. VII) yields not less than 0.8% of natural epinephrine of glandular origin; moisture not more than 6%; ash, not more than 7%. Assayed by the method for Liquor Epinephrinæ Hydrochloridi, *U.S.P. XII*, using an extract of 0.5 g. in 50 ml. of N/10 hydrochloric acid.

Biological Methods of Determination.

Biological methods for the estimation of adrenaline are not required in any of the pharmacopœias since adrenaline is a chemical substance with well-defined chemical properties. Nevertheless biological tests are often applied to adrenaline to determine whether a particular sample is entirely pure or whether its activity corresponds, for example, to only 90% of that of the pure substance. The estimation of adrenaline on the blood pressure of the spinal cat is sufficiently accurate to detect differences of 5 or 6%.

(a) *Estimation of the blood pressure.* A cat is anaesthetised with chloroform and the spinal cord is exposed by removing the laminae of the second cervical vertebra. The spinal cord is divided and the brain is destroyed by passing a probe through the foramen magnum. The anaesthetic can then be discontinued, though the circulation is only maintained so long as artificial respiration is given. The blood pressure is recorded by placing a cannula in the carotid artery, and a cannula is placed in the jugular vein to give injections. In the cat so prepared the blood pressure is low and steady, and well suited for comparing the pressor (blood pressure raising) effect of different doses of adrenaline. A sample of pure adrenaline is taken as standard and a solution of strength 1 in 40,000 in saline acidified with hydrochloric acid is prepared. A similar solution of the unknown sample is prepared. Doses of 0.3 or 0.4 ml. are then injected, using the standard and the unknown alternately, in order to discover what amount of the unknown produces the same effect as a given amount of the standard.

(b) *Estimation on the isolated intestine.* A strip of the intestine of the rabbit is suspended in Ringer's solution; one end is fixed and the other is attached by a thread to a lever so that the movements of the strip are recorded by the lever on a smoked drum. In Ringer's solution supplied with oxygen and maintained at 37°, the strip alternately contracts and relaxes. If adrenaline is added to the bath the contractions are lessened or abolished according to the dose; when the adrenaline is removed the contractions begin again. An unknown solution of adrenaline can be compared with a known solution by using this property of inhibiting the movements.

Ewin's Colorimetric Test. Potassium persulphate is added to produce a concentration of 0.1% and the mixture placed in a boiling water-bath. A red colour is produced. This shows adrenaline in a dilution of 1 in 5 million.—H. Dryerre, *Chem. & Drugg.*, i/1922, 418.

Colorimetric determination in extracts of suprarenal glands gives results comparable with the biological test. Gland extracts at pH 5.4 and containing 0.01 to 0.1 mg. of adrenaline are mixed with an equal volume of the reagent containing potassium persulphate, 0.2%; sodium chloride, 1%; sodium phosphate, 0.239%; and sodium acid phosphate, 0.937%. The increase in red colour, determined with a tintometer, during 30 minutes at 22° is proportional to the adrenaline content.—J. H. Barker, C. J. Eastland and N. Evers, *Biochem. J.*, 1932, 2129.

Errors in the above test are due to the presence of ascorbic acid and uric acid, chiefly the former. Good agreement is found between the figure found by the pressor assay and that obtained by the colorimetric method if allowance is made for the ascorbic acid, determined by indophenol titration, and uric acid determined by the Folin method after precipitation with silver lactate and re-dissolving in lithium chloride, sodium cyanide and water.—J. Devine, *Biochem. J.*, 1937, 545.

Mercuric salts with adrenaline give a definite colour reaction which has been used for analysis of adrenaline contained in medicinal preparations. Details are given for the determination of adrenaline in adrenal extracts for injection, powdered gland, fresh gland and solution of adrenaline (1 in 1000), *Fr. Cx.* The results obtained compare closely with those given by physiological assays.—P. Bouvet, *J. Pharm. Chim., Paris*, 1939, 29, 481.

Extract of Suprarenal Cortex.

Biological Method of Assay. The potency of extracts of suprarenal cortex was first demonstrated by showing that they were able to prolong the life of cats or dogs from which the suprarenal glands were removed. This method is still used, but it suffers from the drawback that several animals are needed for testing each extract, and after the animals have been kept for 3 weeks from the time of removal of the glands it is uncertain whether some small portion of gland has regenerated or not. Very commonly rats are used, since, provided they are young,

they die after removal of the suprarenals. Schultzer has described a method (*J. Physiol.*, 1936, 87, 222) in which two preparations can be compared by injecting them daily for 21 days into groups of adrenalectomised rats. The percentage of rats which survive to the end of this period depends on the dose injected, and it is possible to discover what dose of each preparation will prolong the life of 50 per cent. of rats for 21 days. The doses of the two preparations which have this effect can then be equated.

The most rapid and the best method from the point of view of accuracy of estimating extract of suprarenal cortex is that described by Bülbring, *J. Physiol.*, 1937, 89, 64. The suprarenal glands are removed from groups of drakes. The mean survival time of uninjected birds is about 8 hours. If, however, after the operation birds are injected hourly with cortical extract, the survival time can be prolonged indefinitely. Bülbring has shown that the relation between the logarithm of the dose injected and the survival time is linear, and has determined the equation to the regression line. If a comparison of two preparations is made using 10 birds for each, the standard deviation of the comparison is 22 per cent. Since Bülbring recommends that the injections be made for a period of 20 hours, the test may be completed in one day. The operations, however, require considerable skill.

A rapid method of assay for suprarenal cortical extract is described. It is based on the increase in potassium excretion after subcutaneous injection of the extract in rats maintained on a diet of constant potassium content. For routine use four groups of four rats are used, two of these groups being injected with one dose of one extract and two with one dose of the other. The potencies can then be calculated, using the formula for the regression line, and the whole can be completed within ten days.—G. B. West, *Quart. J. Pharm.*, 1941, 26.

A method of assay for the adrenal cortical hormone based on the use of the month-old adrenalectomised rat, and a method of preparation of concentrates. The final biologically active fraction, which is practically free from N, will maintain adrenalectomised rats in daily doses of 0.1 mg., equivalent to 10 g. of whole ox adrenal gland.—G. Hunter and M. M. Cantor, *Canad. med. Ass. J.*, ii/1937, 368.

Fr. Cx. 1937. Assay of Poudre de Glande Surrénale. Shake 1 g. with 10 ml. N/10 sulphuric acid for 15 minutes and dilute to 100 ml.; to 1 g. sodium acetate in 8 ml. water add 2 ml. of the solution followed by 3 drops of mercuric chloride (5%) solution; after 3 minutes match the red coloration against a standard solution. The powder should contain at least 1% of adrenaline by the colorimetric method and by biological test.

Desoxycorticosteroni Acetas (*B.P.C. Supp. III*). $C_{23}H_{33}O_4$ = 372.25. M.p., 154° to 159° . α_D of 1% solution in dehydrated alcohol, $+175^\circ$ to $+185^\circ$. Loss at 100° , not more than 0.5%. Ash, not more than 0.1%.

ÆTHER

Æther (*B.P. Add. I*). $(C_2H_5)_2O$ = 74.08. The boiling-range should be 34° to 36° and the specific gravity should be from 0.720 to 0.724. It should comply with limit tests for peroxides and residue on evaporation. Water heated with it should have a pH value of not less than 4.9. Complies with the test for methyl alcohol in Æther Anæstheticus. Æther, *U.S.P. XII*, containing 96 to 98% of $(C_2H_5)_2O$, boils at about 35° and has a sp. gr. of 0.713 to 0.716 at 25° . Tests for acidity, residue, foreign odour on evaporation, aldehyde and peroxide are specified. Æthylis Oxidum, *U.S.P. XII*, a solvent ether containing 96 to 98% of $(C_2H_5)_2O$, conforms to less stringent tests for peroxide and aldehydes.

A British Standard Specification (B.S.S. No. 579—1934) has been issued by the British Standards Institution for Technical Ether. The specification includes requirements regarding description, sp. gr. (not exceeding 0.725 at 15.5°C), distillation, residue on evaporation, acidity, peroxides (titanium oxide test), sulphur compounds and sampling, and the appendices describe the methods and apparatus to be used.

A method, accurate to $\pm 1\%$, is described for the determination of diethylether in preparations such as simple collodion, ethereal tincture of lobelia and spirit of ether.—E. J. Boorman, *J. Soc. chem. Ind., Lond.*, 1938, 65.

An official method for the determination of ether, inapplicable in the presence of essential oils, is described in *Methods of Analysis (A.O.A.C., 1940, 604)*.

Acetone in Ether. A sample of ordinary 0.720 ether from S.V.M. gave nearly 24 parts of acetone per 10,000. As a qualitative test **Rothera's Nitro-Prusside Test** may be used. 5 ml. of ether, 1 ml. of 5% sodium nitro-prusside solution and 3 ml. of strong ammonia are shaken together. Then solid ammonium chloride is added, *q.s.* to supersaturate, and the whole shaken. Samples will show a slight reaction or none at all.—A. J. Jones, *Yearb. Pharm.*, 1919, 403.

Scott Wilson's Reagent. Dissolve mercuric cyanide, 0.5 g., and sodium hydroxide, 9 g., in water, 60 ml., and add with constant stirring 20 ml. of 0.727% silver nitrate solution. No turbidity should develop on shaking the ether with excess of the reagent.

For quantitative determination employ Scott Wilson's method.—*J. Physiol.*, 1907, 444.

Æther Anæstheticus (B.P.). The boiling-range is limited to 34° to 35°. No foreign odour is perceptible on evaporation; no brown or red colour of peroxides is produced when shaken with potassium iodide and starch solution in a completely filled bottle and set aside in the dark for half an hour; presence of acetone and aldehyde is eliminated by no colour or turbidity being produced when shaken and set aside with Nessler's reagent; and no reaction for methanol is given by water shaken with it. *Æther, U.S.P. XII*, is only to be used for anæsthesia when preserved in small well-closed containers and the original container must not have been opened more than 24 hours.

The difference between *Æther* and *Æther Anæstheticus* is the difference between two fractions from the same distillation. Anæsthetic ether is prepared from a fraction containing less than 0.5% of alcohol. Ether is prepared from fractions containing more than 0.5% of alcohol, a denaturant, usually 5% of wood spirit, being added. Only anæsthetic ether should be official.—W. H. Linnell, *Pharm. J.*, i/1933, 106.

Peroxides in Anæsthetic Ether. Decomposition, with formation of peroxides, prevented by addition of $\frac{1}{2}$ grain of hydroquinone to 4 oz. of ether. One part of hydroquinone to 5000 parts of ether is sufficient.—H. O. Nolan, *Lancet*, ii/1933, 129.

Powdered iron, 1 g. per 100 ml. of freshly distilled ether, prevents formation of peroxides.—*Pharm. J.*, ii/1927, 434.

JORRISON'S REAGENT (0.4 g. of vanadic acid in 4 ml. of sulphuric acid and 96 ml. water) gives a red colour with ether if peroxides are present. Aldehydes and unsaturated alcohols give a blue colour on keeping.—*J. chem. Soc. Abstr.*, ii/1924, 706.

Ferrous Thiocyanate Test. To ether, 5 ml., add N/10 potassium thiocyanate solution, 1 ml., and fresh 5% ferrous ammonium sulphate solution, 1 drop. If peroxide be present a pink or red tinge develops almost immediately.—F. H. Hocking, per *Chem. & Drugg.*, i/1927, 592. See also *Yearb. Pharm.*, 1924, 615.

Determination of minute quantities of peroxides. The following method is stated to be accurate for less than one part per million of ethyl peroxide. Place 10 ml. of the sample and 20 ml. of dehydrated alcohol in a 250 ml. stoppered flask, and add a freshly prepared solution of cadmium iodide, 1 g., and potassium iodide, 1 g., in 5 ml. of acetic acid (36%). Mix thoroughly and allow to stand

for one hour in the dark. Conduct a blank experiment on the reagents simultaneously. If any yellow tint is visible, titrate with N/50 sodium thiosulphate (prepared with CO_2 -free distilled water). Towards the end of the titrations stopper the flasks and shake vigorously. Each ml. of N/50 sodium thiosulphate is equivalent to 9.008 parts of $(\text{C}_2\text{H}_5)_2\text{O}_2$ per million.—L. W. Green and R. E. Schoetzow, *J. Amer. pharm. Ass.*, 1933, 412.

Æthylenum (B.P.). $\text{C}_2\text{H}_4 = 28.03$. Not more than 2% by volume of gas remains when a volume equivalent to 1000 to 1500 ml. is passed into a gas pipette containing fuming sulphuric acid or bromine solution and the residual gas treated with potassium hydroxide solution, corresponding to not less than 98% by volume of C_2H_4 ; absence of carbon monoxide in the gas remaining is shown by no further contraction of volume occurring, after treating first with alkaline solution of pyrogallol, when it is treated with freshly prepared acid cuprous chloride solution. Tests for limit of carbon dioxide, acid and sulphur dioxide, acetylene, phosphine, aldehydes and hydrogen sulphide and carbon monoxide are included. *Æthylenum, U.S.P. XII*, leaves not more than 1% by volume unabsorbed by bromine solution and potassium hydroxide solution, corresponding to 99% v/v of C_2H_4 ; 2000 ml. does not turn 100 ml. of boiled and cooled water, with methyl red and 0.2 ml. of N/100 hydrochloric acid, more red than 100 ml. of the water and methyl red with 0.4 ml. of N/100 hydrochloric acid, nor more yellow than 100 ml. of the water with methyl red. A test for limit of carbon dioxide is included. Tested for limit of carbon monoxide by addition of 2.5 ml. of diluted blood to 250 ml. of ethylene and agitation for 15 minutes; after addition of 0.02 g. each of pyrogallol and tannic acid, shaking and standing in the dark for 15 minutes, no pink colour should be observed and the grey colour produced must match that produced by similarly treating 250 ml. of air which has been collected remote from sources of carbon monoxide.

Cyclopropanum (U.S.P. XII). $\text{C}_3\text{H}_6 = 42.05$. Contains 99% v/v of C_3H_6 , by measurement of the residual gas after absorption in sulphuric acid. Limits for acids and alkalis, carbon dioxide, propylene, allene, and other unsaturated hydrocarbons and of halogens are included.

ÆTHYLIS CHLORIDUM

Æthylis Chloridum (B.P.). $\text{C}_2\text{H}_5\text{Cl} = 64.50$. Contains not less than the equivalent of 99.5% w/w of $\text{C}_2\text{H}_5\text{Cl}$. The assay by digestion with N/2 alcoholic potassium hydroxide in a water-bath for thirty minutes is incomplete; it is best conducted by introducing about 1.5 g. into a stoppered bottle containing 50 ml. of N/1 alcoholic potassium hydroxide, weighed accurately, and heating in a water-bath for at least 1 hour; titrate the excess of alkali with N/2 hydrochloric acid to phenolphthalein; conduct a blank experiment for an identical time using a bottle of exactly similar glass. During evaporation no foreign odour is at any time detectable and the residue is not more than 0.01%. Tests for

limit of acidity, alkalinity, ionisable chlorides and ethyl alcohol are included. *Æthylis Chloridum, U.S.P. XII*, is not assayed; tests for chloride, neutrality, alcohol, foreign volatile matter and residue on evaporation are included.

AGAR

Agar (*B.P.*). Yields not more than 5% of ash. It should be soluble when boiled with 100 parts of water, yielding a stiff jelly on cooling. Should yield no precipitate on addition of tannic acid to a hot 0.2% solution. 10 ml. of a rapidly cooled 0.2% aqueous solution gives a pale yellow colour with one drop of N/10 iodine and a dark purple coloration with 0.5 ml.; on setting aside for two hours, 0.5 ml. of the iodine solution gives a brownish colour. The *U.S.P. XII* substance contains not more than 1% of foreign organic matter; acid-insoluble ash, not more than 1%; moisture limit, 20% when determined by the toluene distillation method. A solution of 0.1 g. in 100 ml. of boiling water, on cooling, gives no blue colour with iodine. A 1% solution in boiling water, cooled to 50°, gives no turbidity within 10 minutes with an equal volume of picric acid solution.

Tests for Identity. The following tests for the identity of agar are recommended by the Sub-Committee on Crude Drugs of the Committee on Pharmacy and Pharmacognosy:—To 4 ml. of a 0.5% *w/v* solution in water, add 0.5 ml. of hydrochloric acid and heat for thirty minutes in a water-bath. Divide the liquid into two parts. To one part add 1.5 ml. of solution of sodium hydroxide and 3 ml. of solution of potassio-cupric tartrate and warm in a water-bath; a red precipitate is produced. To the remainder of the liquid add solution of barium chloride; a white precipitate is produced (distinction from tragacanth). When powdered it acquires a pink colour in solution of ruthenium red (distinction from acacia and tragacanth). On addition of N/50 iodine it acquires a deep crimson colour (distinction from acacia and from tragacanth).—(*British Pharmacopœia Commission Report*, No. 13, September 1939).

ALCOHOL ÆTHYLICUM

Alcohol (*B.P.*). Contains from 94.7 to 95.2% *v/v* or 92.0 to 92.7% *w/v* of C_2H_5OH . Sp. gr., 0.815 to 0.817. Refractive index at 20°, 1.3637 to 1.3639. Limit tests for acidity, alkalinity, oily or resinous substances, fusel oil and allied impurities, and aldehyde are included. Absence of methyl alcohol is shown by the *B.P.* test: 5 ml. of a 10% aqueous dilution is mixed with 2 ml. of a solution of potassium permanganate in phosphoric acid and set aside for 10 minutes, after which 2 ml. of solution of oxalic and sulphuric acids is added and to the colourless solution 5 ml. of decolorised magenta solution is added; no colour is produced within 10 minutes. Industrial methylated spirit gives a deep violet coloration within 5 minutes by this test. Residue on evaporation and drying at 100°, not more than 0.01%

w/v. The official alcohol of the *U.S.P. XII* contains not less than 92.3% *w/w* or 94.9% *v/v* of C_2H_5OH at 15.56°; residue on evaporation on a water-bath and drying at 100°, not more than 0.0025%. Tests for acidity, fusel oil constituents, amyl alcohol or non-volatile carbonisable substances, aldehydes and organic impurities, methanol and acetone, ketones, tertiary butyl alcohol and isopropyl alcohol, alkaloids and for formaldehyde are also included.

Alcohol Dehydratum (B.P.). $C_2H_5OH=46.05$. Has a sp. gr. of 0.7936 to 0.7967, a refractive index at 20° of 1.3614 to 1.3618 and contains not less than 99.4% by volume or 99% by weight of C_2H_5O . Alcohol Dehydratum, *U.S.P. XII*, containing 99% by weight of C_2H_5OH , has sp. gr. not higher than 0.798 at 15.56°.

A British Standard Specification (B.S.S. No. 507—1933) has been issued by the British Standards Institution for ethyl alcohol. The specification includes requirements regarding description, strength, miscibility with water, residue on evaporation, acidity, aldehyde content and sampling, and the appendices describe the methods and apparatus to be used. This alcohol is 66° O.P. (91.95% by weight or 94.68% by volume) or from 61° to 68° O.P. (91.83 to 95.81% by volume) if agreed between purchaser and vendor.

The strength of alcohol is usually expressed in terms of vol. %, though the Board of Customs and Excise favour "proof" terms (see Vol. I, p. 156).

Determination in Aqueous Dilutions by Capillary Rise. A method is described which is based on the variation of surface tension and density as observed by the capillary rise measurement. The sensitivity of the method is greatest when the percentage of alcohol is between 0 and 5, and if the proportion of alcohol is greater than 15% it should be diluted. The average error is then not greater than 0.05%.—F. Todd, *Amer. J. Pharm.*, 1936, 488.

Identification in Mixtures. The liquid is distilled and the distillate treated successively with a strong oxidising agent such as potassium hypochlorite in alkaline solution, and then ammoniacal silver nitrate solution and finally redistilled first in alkaline and secondly in acid solution; by this process the ethyl alcohol and its higher homologues are obtained free from other organic substances. The distillate is then oxidised quantitatively with potassium dichromate and the acid formed isolated, measured and identified. If the acid found after oxidation is identified as acetic acid, and if the amount of oxygen required, measured by the amount of potassium dichromate used, is the same as that which would theoretically be required to produce the same amount of acetic acid from ethyl alcohol, the liquid can only be ethyl alcohol. The identification of propionic, butyric or valeric acid indicates the presence of the corresponding alcohol. Butyl alcohol on oxidation yields both acetic and butyric acids, in the proportion of six parts of butyric acid to two parts of acetic acid, and thus the presence of acetic acid in the oxidation mixture does not specifically show the presence of ethyl alcohol. In a mixture of ethyl and butyl alcohols, by determining the respective quantities of the two acids formed on oxidation and subtracting the proportion of acetic acid produced from the butyl alcohol, the quantity of acetic acid produced from the ethyl alcohol is obtained, and the original amount of ethyl alcohol may be calculated.—M. Nichoux, *C. R. Acad. Sci., Paris*, 1936, 203, 16.

ALCOHOL DILUTION RULES

If *V* be the volume percentage of the stronger alcohol and *v* that of the alcohol required—

I. *By volume.* Mix *v* volumes of the stronger alcohol with distilled water, q.s., after cooling to make *V* volumes, e.g. to make an alcohol 43% from alcohol 95% take 43 volumes of the 95% and make up to 95 volumes. (See also Appendix III, *B.P.C.* '34, for temperature correction tables.)

II. *By weight.* Proceed on the same lines by weight throughout.

To Transpose Volume per cent. of Alcohol into Weight per cent.
The volume per cent. is multiplied by 0.7938, and the product divided by the sp. gr. of the liquid, e.g., 80.22% v/v = $\frac{80.22 \times 0.7938}{0.863} = 73.7875\%$ w/w.

To express the weight per cent. as volume per cent. divide the weight per cent. by 0.7938 and multiply by the sp. gr. of the liquid, e.g., 90.29% by weight = $\frac{90.29 \times 0.822}{0.7938} = 93.49\%$ v/v.

Proof Spirit Conversion Factors (F. C. J. Bird, *Chem. & Drugg.*, Dec. 27 1919).

METRIC MEASURE.

To express:—

| | | | | | |
|-----------|-----|-------------------------------|----|-------------|--------|
| Litres of | 90% | Alcohol B.P. in PROOF GALLONS | .. | Multiply by | 0.3471 |
| " | 70% | " | " | " | 0.2697 |
| " | 60% | " | " | " | 0.2312 |
| " | 45% | " | " | " | 0.1733 |
| " | 20% | " | " | " | 0.0767 |

IMPERIAL MEASURE.

| | | | | | |
|---------|-----|-------------------------------|----|-------------|--------|
| Gallons | 90% | Alcohol B.P. in PROOF GALLONS | .. | Multiply by | 1.5779 |
| " | 70% | " | " | " | 1.2263 |
| " | 60% | " | " | " | 1.051 |
| " | 45% | " | " | " | 0.7877 |
| " | 20% | " | " | " | 0.3487 |

QUANTITY OF SPIRITS WHICH MAY BE SOLD BY A CHEMIST WITHOUT LICENCE.

In view of the Immature Spirits (Restrictions) Act, 1915, the Commissioners of Customs and Excise reduced the quantity of duty-paid Spirits of Wine (including Absolute Alcohol), which may be sold without a licence, to 5 ounces fluid. Strict inquiries should be made by the pharmacist as to requirements. —*Pharm. J.*, i/1922, 505.

To State Volume per cent. as Alcohol of Proof Strength. Multiply V per cent. by 1.753 and deduct 100 from the product. Thus 65% v/v = $65 \times 1.753 - 100 = +13.945^\circ$ over proof. Further, alcohol of 25% v/v = $25 \times 1.753 - 100 = -56.175^\circ$ proof, i.e., 56.175° under proof.

(B.P. 1885 stated: Proof spirit=about 57% C_2H_5OH by vol., i.e., 57 parts alcohol with water produce 100 parts proof spirit.)

∴ 1 part pure alcohol = $\frac{100}{57} = 1.753$ (about) parts proof strength.

Conversely, to State Alcohol of Proof Strength as Volume per cent.:—

Add 100 to the proof strength and divide the product by 1.753; thus,

$$13.945^\circ \text{ o.p.} = \frac{113.945}{1.753} = 65\% \text{ by vol., and}$$

$$56.175^\circ \text{ u.p.} = \frac{100 - 56.175}{1.753} = 25\% \text{ by vol.}$$

To Convert Bulk Gallons to Proof Gallons multiply by

$$\frac{\text{Proof Strength} + 100}{100}$$

100 vols. of alcohol 90% (approx. 58° o.p.) are equivalent to 153 vols. of proof spirit.

"Proof Spirit" has sp. gr. 0.920. This, formerly, was found to be the weakest spirit that could be put to the proof of igniting a little gunpowder moistened with it. If the spirit caught fire and inflamed the gunpowder, it was designated "over proof," and if not, "under proof." By the Hydrometer Act, 58 Geo. III., cap. 28, Proof Spirit is defined as spirit of strength which, at a temperature of 51°F., weighs exactly twelve-thirteenths of an equal quantity of distilled water.

The following Table, founded on B.P. 1898 and Gilpin's Tables, shows:—

(i) The volume of distilled water necessary to be added to 100 volumes of alcohol (90%) for the production of each strength of diluted alcohol.

(ii) The volumes of alcohol (90%), and of distilled water respectively which, when mixed and reduced to 60°F. (15.5°C.), will produce, allowing for contraction in volume, 1000 ml., 1 pint, or 1 gallon of each strength of diluted alcohol.

The sp. gr. and the exact Excise (Sikes') strength at 60°F. (15.5°C.), in degrees over proof (O.P.) and under proof (U.P.), of each dilution, are given in the first column.

Table for the Dilution of Alcohol (90%) to Weaker Strengths

| Volume Percentage, Specific Gravity, and Excise Strength | Alcohol (90%) | Distilled Water | Volume Produced |
|--|--|--------------------|--|
| 70 % Sp. gr. 0.8900 22.7 O.P.† | 100 vols. + 31.05 vols. 777.8 ml. + 241.6 ml. *648.5 g. + 241.6 g. 15 oz. 266 m. + 4 oz. 398 m. 124 oz. 215 m. + 38 oz. 307 m. *6 lbs. 7½ oz. + 2 lbs. 6½ oz. | | = 128.57 vols. = 1000 ml. = 1000 ml. = 1 pint = 1 gal. = 8 lbs. 14½ oz. |
| 60 % Sp. gr. 0.9135 5.20° O.P.† | 100 vols. + 53.65 vols. 666.7 ml. + 357.8 ml. *555.9 g. + 357.8 g. 13 oz. 160 m. + 7 oz. 74 m. 106 oz. 320 m. + 57 oz. 112 m. *5 lbs. 9 oz. + 3 lbs. 9½ oz. | | = 150 vols. = 1000 ml. = 1000 ml. = 1 pint = 1 gal. = 9 lbs. 2½ oz. |
| 45 % Sp. gr. 0.9436 21.2° U.P.† | 100 vols. + 105.34 vols. 500 ml. + 526.6 ml. *417.2 g. + 526.6 g. 10 oz. + 10 oz. 256 m. 80 oz. + 84 oz. 130 m. *4 lbs. 2½ oz. + 5 lbs. 4½ oz. | | = 200 vols. = 1000 ml. = 1000 ml. = 1 pint = 1 gal. = 9 lbs. 7 oz. |
| 20 % Sp. gr. 0.9760 64.9° U.P.† | 100 vols. + 355.8 vols. 222.2 ml. + 790.7 ml. *185.2 g. + 791 g. 4 oz. 213 m. + 15 oz. 390 m. 35 oz. 267 m. + 126 oz. 243 m. *1 lb. 13½ oz. + 7 lbs. 14½ oz. | | = 450 vols. = 1000 ml. = 1000 ml. = 1 pint = 1 gal. = 9 lbs. 12½ oz. |

NOTE.—*These figures are the weights necessary to produce a gallon and a litre respectively, at 15.5°C. † Stevenson.

Amount of Ethyl Alcohol by Volume in Various Liquors.

| | | | |
|-------------------------------|-----------|------------------------|--------------|
| Whisky | 51 to 59% | White Wine | 12 to 14% |
| Rum | | Champagne | 10 to 13% |
| Gin | | Orange Wine | 10 to 12% |
| Strong Liqueurs .. | | Burgundy | 9 to 12% |
| Proof Spirit .. 57 | | Hock | 9 to 12% |
| Brandy 43 to 57% | | Claret | 8 to 12% |
| Port 20 to 30% | | Cider | 5 to 9% |
| White Wine (strong) 23 to 29% | | Strong Ale or Stout .. | 5 to 9% |
| Sherry 16 to 22% | | Beer and Porter | 2 to 5% |
| Madeira 16 to 22% | | | —HALE WHITE. |

The current strengths (pre-war) are: Whisky, Rum, Gin and Brandy, 40 to 47%; Strong Liqueurs, 43 to 47%; Proof Spirit, 57·1%; Port, 16 to 22%; Sherry, 16 to 22%; Madeira, 16 to 22%; White Wine, 12 to 14%; Champagne, 10 to 13%; Orange Wine, 12 to 15%; Burgundy, 12 to 14%; Hock, 12 to 14%; Claret, 10 to 14%.

ETHYL ALCOHOL TABLE.

As employed in the Government Laboratory.

| Sp.Gr. at 15·5°C. | Wt. per cent. | Vol. per cent. | Sp.Gr. at 15·5°C. | Wt. per cent. | Vol. per cent. | Sp.Gr. at 15·5°C. | Wt. per cent. | Vol. per cent. | Sp.Gr. at 15·5°C. | Wt. per cent. | Vol. per cent. |
|-------------------------|---------------------|----------------------|-------------------------|---------------------|----------------------|-------------------------|---------------------|----------------------|-------------------------|---------------------|----------------------|
| 0·999 | 0·53 | 0·66 | 0·947 | 36·00 | 42·95 | 0·895 | 60·23 | 67·92 | 0·843 | 82·00 | 87·09 |
| 0·998 | 1·07 | 1·34 | 0·946 | 36·54 | 43·54 | 0·894 | 60·66 | 68·33 | 0·842 | 82·40 | 87·42 |
| 0·997 | 1·61 | 2·02 | 0·945 | 37·07 | 44·13 | 0·893 | 61·09 | 68·74 | 0·841 | 82·80 | 87·74 |
| 0·996 | 2·17 | 2·71 | 0·944 | 37·60 | 44·71 | 0·892 | 61·52 | 69·14 | 0·840 | 83·20 | 88·06 |
| 0·995 | 2·73 | 3·42 | 0·943 | 38·12 | 45·28 | 0·891 | 61·95 | 69·55 | 0·839 | 83·60 | 88·37 |
| 0·994 | 3·31 | 4·14 | 0·942 | 38·64 | 45·85 | 0·890 | 62·38 | 69·95 | 0·838 | 83·99 | 88·68 |
| 0·993 | 3·90 | 4·88 | 0·941 | 39·15 | 46·40 | 0·889 | 62·81 | 70·35 | 0·837 | 84·39 | 88·99 |
| 0·992 | 4·51 | 5·63 | 0·940 | 39·65 | 46·95 | 0·888 | 63·24 | 70·75 | 0·836 | 84·78 | 89·30 |
| 0·991 | 5·13 | 6·40 | 0·939 | 40·15 | 47·50 | 0·887 | 63·67 | 71·15 | 0·835 | 85·17 | 89·61 |
| 0·990 | 5·76 | 7·18 | 0·938 | 40·65 | 48·04 | 0·886 | 64·10 | 71·55 | 0·834 | 85·56 | 89·91 |
| 0·989 | 6·41 | 7·98 | 0·937 | 41·15 | 48·57 | 0·885 | 64·53 | 71·95 | 0·833 | 85·95 | 90·22 |
| 0·988 | 7·08 | 8·80 | 0·936 | 41·64 | 49·10 | 0·884 | 64·96 | 72·34 | 0·832 | 86·34 | 90·52 |
| 0·987 | 7·76 | 9·65 | 0·935 | 42·13 | 49·63 | 0·883 | 65·39 | 72·74 | 0·831 | 86·73 | 90·82 |
| 0·986 | 8·46 | 10·51 | 0·934 | 42·62 | 50·15 | 0·882 | 65·81 | 73·13 | 0·830 | 87·11 | 91·11 |
| 0·985 | 9·13 | 11·40 | 0·933 | 43·11 | 50·67 | 0·881 | 66·24 | 73·52 | 0·829 | 87·50 | 91·40 |
| 0·984 | 9·91 | 12·29 | 0·932 | 43·59 | 51·18 | 0·880 | 66·66 | 73·91 | 0·828 | 87·88 | 91·69 |
| 0·983 | 10·65 | 13·20 | 0·931 | 44·06 | 51·68 | 0·879 | 67·09 | 74·30 | 0·827 | 88·27 | 91·98 |
| 0·982 | 11·42 | 14·13 | 0·930 | 44·53 | 52·18 | 0·878 | 67·51 | 74·68 | 0·826 | 88·65 | 92·26 |
| 0·981 | 12·20 | 15·08 | 0·929 | 45·00 | 52·67 | 0·877 | 67·93 | 75·06 | 0·825 | 89·03 | 92·55 |
| 0·980 | 12·99 | 16·04 | 0·928 | 45·47 | 53·16 | 0·876 | 68·35 | 75·44 | 0·824 | 89·41 | 92·83 |
| 0·979 | 13·80 | 17·02 | 0·927 | 45·94 | 53·65 | 0·875 | 68·77 | 75·82 | 0·823 | 89·79 | 93·11 |
| 0·978 | 14·61 | 18·00 | 0·926 | 46·40 | 54·14 | 0·874 | 69·19 | 76·19 | 0·822 | 90·16 | 93·38 |
| 0·977 | 15·43 | 18·99 | 0·925 | 46·87 | 54·62 | 0·873 | 69·62 | 76·57 | 0·821 | 90·53 | 93·65 |
| 0·976 | 16·25 | 19·98 | 0·924 | 47·33 | 55·10 | 0·872 | 70·04 | 76·94 | 0·820 | 90·90 | 93·92 |
| 0·975 | 17·08 | 20·97 | 0·923 | 47·79 | 55·58 | 0·871 | 70·46 | 77·32 | 0·819 | 91·27 | 94·19 |
| 0·974 | 17·90 | 21·96 | 0·922 | 48·25 | 56·05 | 0·870 | 70·88 | 77·69 | 0·818 | 91·63 | 94·45 |
| 0·973 | 18·72 | 22·94 | 0·921 | 48·71 | 56·52 | 0·869 | 71·30 | 78·06 | 0·817 | 92·00 | 94·71 |
| 0·972 | 19·53 | 23·91 | 0·920 | 49·17 | 56·99 | 0·868 | 71·72 | 78·43 | 0·816 | 92·36 | 94·97 |
| 0·971 | 20·34 | 24·85 | 0·919 | 49·63 | 57·46 | 0·867 | 72·14 | 78·80 | 0·815 | 92·72 | 95·22 |
| 0·970 | 21·14 | 25·83 | 0·918 | 50·08 | 57·92 | 0·866 | 72·55 | 79·17 | 0·814 | 93·08 | 95·47 |
| 0·969 | 21·93 | 26·77 | 0·917 | 50·53 | 58·38 | 0·865 | 72·97 | 79·53 | 0·813 | 93·44 | 95·72 |
| 0·968 | 22·71 | 27·69 | 0·916 | 50·98 | 58·83 | 0·864 | 73·39 | 79·89 | 0·812 | 93·80 | 95·97 |
| 0·967 | 23·48 | 28·69 | 0·915 | 51·43 | 59·29 | 0·863 | 73·81 | 80·25 | 0·811 | 94·15 | 96·21 |
| 0·966 | 24·23 | 29·48 | 0·914 | 51·88 | 59·74 | 0·862 | 74·22 | 80·61 | 0·810 | 94·50 | 96·45 |
| 0·965 | 24·97 | 30·34 | 0·913 | 52·33 | 60·19 | 0·861 | 74·64 | 80·97 | 0·809 | 94·85 | 96·69 |
| 0·964 | 25·68 | 31·18 | 0·912 | 52·77 | 60·63 | 0·860 | 75·05 | 81·32 | 0·808 | 95·20 | 96·93 |
| 0·963 | 26·37 | 31·99 | 0·911 | 53·21 | 61·07 | 0·859 | 75·47 | 81·68 | 0·807 | 95·55 | 97·16 |
| 0·962 | 27·06 | 32·79 | 0·910 | 53·65 | 61·51 | 0·858 | 75·88 | 82·03 | 0·806 | 95·89 | 97·39 |
| 0·961 | 27·73 | 33·58 | 0·909 | 54·10 | 61·95 | 0·857 | 76·30 | 82·38 | 0·805 | 96·23 | 97·62 |
| 0·960 | 28·39 | 34·33 | 0·908 | 54·54 | 62·39 | 0·856 | 76·71 | 82·73 | 0·804 | 96·57 | 97·84 |
| 0·959 | 29·03 | 35·06 | 0·907 | 54·98 | 62·83 | 0·855 | 77·12 | 83·08 | 0·803 | 96·91 | 98·06 |
| 0·958 | 29·66 | 35·79 | 0·906 | 55·42 | 63·26 | 0·854 | 77·53 | 83·42 | 0·802 | 97·25 | 98·28 |
| 0·957 | 30·28 | 36·50 | 0·905 | 55·87 | 63·70 | 0·853 | 77·94 | 83·77 | 0·801 | 97·59 | 98·49 |
| 0·956 | 30·90 | 37·20 | 0·904 | 56·31 | 64·13 | 0·852 | 78·35 | 84·11 | 0·800 | 97·91 | 98·70 |
| 0·955 | 31·50 | 37·89 | 0·903 | 56·75 | 64·56 | 0·851 | 78·76 | 84·44 | 0·799 | 98·24 | 98·91 |
| 0·954 | 32·09 | 38·57 | 0·902 | 57·18 | 64·98 | 0·850 | 79·17 | 84·78 | 0·798 | 98·57 | 99·12 |
| 0·953 | 32·67 | 39·22 | 0·901 | 57·62 | 65·41 | 0·849 | 79·58 | 85·12 | 0·797 | 98·90 | 99·32 |
| 0·952 | 33·25 | 39·87 | 0·900 | 58·06 | 65·83 | 0·848 | 79·98 | 85·46 | 0·796 | 99·22 | 99·52 |
| 0·951 | 33·81 | 40·50 | 0·899 | 58·50 | 66·25 | 0·847 | 80·39 | 85·80 | 0·795 | 99·55 | 99·72 |
| 0·950 | 34·37 | 41·13 | 0·898 | 58·93 | 66·67 | 0·846 | 80·79 | 86·12 | 0·794 | 99·87 | 99·92 |
| 0·949 | 34·92 | 41·74 | 0·897 | 59·37 | 67·08 | 0·845 | 81·20 | 86·44 | 0·79359 | 100·00 | 100·00 |
| 0·948 | 35·46 | 42·35 | 0·896 | 59·80 | 67·50 | 0·844 | 81·60 | 86·77 | | | |

Analysis of Wines. *P. Helv. V* gives a useful summary.

Spirit tables for use with Sikes' A and B Hydrometers, issued by the Commissioners of H.M. Customs and Excise for use in connection with "The Strength and Weight of Spirits Ascertainment Regulations," are available (H.M. Stationery Office).

Alcohol Limits of B.P. Galenicals. Insufficient allowance has been made for moisture in the air-dry drug and for the volume occupied by the solid extractive. Revised limits are suggested for the majority of preparations.—T. T. Cocking, *Quart. J. Pharm.*, 1934, 76.

Presence of Methyl Alcohol in Galenicals. Some official preparations, especially those prepared from orange or lemon peel, contain methyl alcohol, probably derived from decomposition of pectin. The quantity present is sufficient to respond to Denigés' test.—R. W. Richardson, *Pharm. J.*, ii/1936, 249.

Diethyl Phthalate. A British Standard Specification (B.S.S. No. 574—1934) has been issued by the British Standards Institution for diethyl phthalate. The specification includes requirements regarding description, specific gravity (1.114 to 1.129 at 15.5°), refractive index (1.4805 to 1.506 at 20°), water, ash, acidity, ester content (not less than 98% w/w) and sampling, and the appendices describe the methods and apparatus to be used.

DETECTION OF DIETHYL PHTHALATE IN ALCOHOLIC LIQUIDS. The following test of the Bureau of Internal Revenue of the U.S. Treasury Department was found satisfactory:—(a) In presence of considerable amount. To 10 ml. of sample add 2 ml. of 10% NaOH and evaporate to dryness on the steam-bath. Add 5 ml. of conc. sulphuric acid and 0.025 g. of resorcinol and heat for 5 minutes on the steam-bath. Transfer to a test-tube, heat in oil bath at 160° for 5 minutes, cool, pour into excess of alkaline water. A greenish yellow fluorescence (due to fluorescein) is produced. (b) In presence of traces. To not less than 50 ml. add 0.2 ml. of 10% NaOH and proceed as above. Allow the dilute fluorescein solution to stand 24 hours and view through the long axis of a Nessler glass. A blank test on pure alcohol should be made for comparison since a fluorescence is produced in the absence of diethyl phthalate which fades in 24 hours.—J. A. Hardy & L. F. Hoyt, *J. Amer. pharm. Ass.*, 1925, 219.

The following methods are suggested for determination of ethyl phthalate in perfumes or in the presence of essential oils.

As dipotassium phthalate. 1 to 2 g. of sample is refluxed for 1 hour with 25 ml. of N/2 potassium hydroxide in commercial absolute alcohol in a conical flask provided with a cooling tube. The cooling tube is then rinsed with more alcohol and the contents cooled on ice, centrifuged and filtered on a sintered glass filter. The precipitate is washed with 30 to 50 ml. of ice-cold absolute alcohol, heated at 140°, and weighed. 1 g. of precipitate corresponds to 0.917 g. of ethyl phthalate. Other acids giving insoluble potassium salts must be absent. A method is also described in which the potassium salt is converted to the lead salt. A method is also given for determination as lead phthalate.—Y. R. Naves and S. Sabetay, *Bull. Soc. Chim.*, 1937, 5, 102.

Distil a mixture of 25 ml. of sample and 50 ml. of water, collecting 50 ml. of filtrate. Evaporate 10 to 12 ml. of filtrate (or sufficient to correspond to about 5 ml. of absolute alcohol) to dryness on a steam-bath with 5 to 6 drops of sodium hydroxide. When dry moisten with 5 to 6 drops of dilute sulphuric acid to dissolve the sodium salts, add 1.2 ml. of sulphuric acid, rub with a glass rod to ensure complete solution and add to 0.05 g. of resorcinol in a long narrow test-tube. Immerse the tube in an oil-bath at 165° to 175° for from 3½ to 4 minutes; then cool the tube, transfer 0.2 to 0.5 ml. of the acid solution to a 50 ml. separator, add 5 to 10 ml. of water and shake thoroughly with 8 to 10 ml. of ether for 30 seconds. Allow to separate, reject the lower layer, wash the residue three or four times with 10 ml. quantities of water until free from acid, and pour off the ether extract into a long narrow test-tube. To the ether extract add down the side of the tube 2 ml. of 5% sodium hydroxide and allow to stand for 15 minutes. If any diethyl phthalate is present a yellowish-green fluorescence will appear.—I. Schwartz, *J. Amer. pharm. Ass.*, 1936, 749.

Spiritus Frumenti (U.S.P. XII). Contains at 15.56°, 47 to 53% v/v of C₂H₅OH and must have been kept in charred wood containers for not less than 4 years. Complies with tests for glycerin, sugar, etc., indication of storage in wood barrels, acidity, esters, acetone, other ketones, isopropyl alcohol and

tertiary butyl alcohol, for methanol, alkaloids, caramel and some coal tar dyes, and for formaldehyde.

Spiritus Vini Vitis (*U.S.P. XII*). Contains at 15-56°, 48 to 54% *v/v* of C_2H_5OH , and is stored in wood barrels for at least 4 years; complies with a test for acidity and other tests given under *Spiritus Frumenti, U.S.P. XII*.

Alcohol Tribromoethylicum (*B.P. Add. III*). $CBr_3CH_2OH = 282.8$. M.p., 79° to 81°. Presence of dibromoacetaldehyde is limited by adding phenylhydrazine acetate solution to a 2% solution in water when no precipitate forms during 30 minutes; limits of readily carbonisable substances, acidity, ionised halogens sulphates, and heavy metals. Leaves on incineration not more than 0.05% of residue. Contains not less than 99% CBr_3CH_2OH ; assayed by refluxing for 2 hours with N/1 sodium hydroxide, followed by titration of the bromide with excess silver nitrate solution and ammonium thiocyanate. Assayed similarly, after drying for 18 hours over sulphuric acid, Tribromoethanol, *U.S.P. XII*, complies with the same standard for purity. M.p., 79° to 82°, loss on drying, not more than 1%, ash not more than 0.1%.

Bromethol (*B.P. Add. III*) is a 66.7% *w/v* solution of tribromoethyl alcohol in amylene hydrate. *Liquor Tribromoethanolis, U.S.P. XII*, is a solution in the same solvent containing 99 to 101% *w/v* of $C_2H_5Br_3O$.

Vinum Xericum (*B.P.C.*). Contains not less than 16% *v/v* of ethyl alcohol. Sp. gr., 0.990 to 1.000. Total acids as tartaric acid ($C_4H_6O_6$) not less than 0.4%. It contains not more than 450 parts per million of sulphur dioxide, to comply with the Public Health (Preservatives in Food) Regulations. The alcohol content is determined by Method I of the British Pharmacopœia:

Dilute 25 ml. with 100 to 150 ml. of water in a 500 to 800 millilitre flask; add a little pumice powder; connect by a still-head to a condenser and distil at least 90 ml.; dilute with water to 100 ml. at the same temperature as that at which the original volume was measured, determine the sp. gr. and refractive index and by reference to tables calculate the percentage of ethyl alcohol in the original wine; if the refractive index differs by more than 0.0002 from that corresponding with the specific gravity found, saturate 75 ml. with sodium chloride and shake for several minutes with 100 ml. of light petroleum (b.p. 50° to 60°); after standing for half an hour run off the lower layer and wash the petroleum layer with 25 ml. of brine; distil the mixed brine liquids after making just alkaline with normal sodium hydroxide to solid phenolphthalein and determine specific gravity and refractive index as before. Total acids are titrated with fifth normal sodium hydroxide to phenolphthalein.

Sulphur dioxide may be determined by boiling for 10 minutes 500 ml. of water and 20 ml. of hydrochloric acid in a round-bottomed litre flask, which is connected with a water-cooled reflux condenser of which the upper end connects with two absorption flasks in series each containing 10 ml. of hydrogen peroxide solution, and maintaining a current of carbon dioxide (passed through sodium carbonate solution) through the apparatus during the whole process; the contents of the flask are cooled and 50 to 100 grammes of the wine introduced as quickly as possible and boiled for 45 minutes; the absorption flasks are disconnected and the contents titrated with N/10 sodium hydroxide using bromophenol blue as indicator, and subtracting the amount of standard sodium hydroxide required to neutralise 20 ml. of the hydrogen peroxide solution to bromophenol blue.

ALCOHOL AMYLICUM

Alcohol Amylicum (*B.P.C.*). $C_5H_{12}O = 88.09$. Boiling-range 128° to 132° . Sp. gr., 0.815 to 0.817. Acidity as $CH_3 \cdot COOH$, not more than 0.01%.

A *B.S.S.* (No. 696, Part 2, 1936) has been prepared by the British Standards Institution for amyl alcohol for use in the Gerber test.

Iso-Amyl Butyrate. $CH_3 \cdot CH_2 \cdot CH_2 \cdot COO \cdot CH_2 \cdot CH_2 \cdot CH < \begin{smallmatrix} CH_3 \\ CH_2 \end{smallmatrix} = 158.144$. Colourless liquid with sp. gr. 0.882 at 0° . Used as a flavouring agent. Commercially the article contains 78 to 93%; sp. gr. ranges from 0.853 to 0.860. Refractive index, 1.4073 to 1.4110 at 20° . B.p. about 135° .

Amylis Nitris (*B.P.*). $C_5H_{11}O_2N = 117.1$. Contains not less than 90% *w/w* of nitrites, calculated as $C_5H_{11}O_2N$. At least 85% distils between 90° and 100° . Sp. gr., 0.874 to 0.884. Assayed by the interaction of an alcoholic dilution with potassium iodide solution and dilute sulphuric acid in a brine-charged nitrometer, and measurement of the nitric oxide produced; at 15.5° and normal pressure each millilitre of moist nitric oxide is equivalent to 0.0049 g. of $C_5H_{11}O_2N$. Amylis Nitris, *U.S.P. XII*, contains not less than 90% of $C_5H_{11}ONO$, when assayed by the general method for nitrites; carbon dioxide is passed through a solution of potassium iodide in boiling deaerated water, while cooling for three minutes, after adding a dilute hydrochloric acid (1 in 2) (any iodine liberated being just decolorised with N/10 sodium thio-sulphate) and while a dilution of the amyl nitrite in aldehyde-free alcohol is introduced into the mixture; the liberated iodine is then immediately titrated with sodium thiosulphate, all additions being made through an outlet tube to the flask employed, of specified dimensions. Amylium nitrosum, *P. Helv. V*, is assayed by decomposing the amyl nitrite by shaking for 5 minutes 0.5 g. in 10 ml. of alcohol with 20 ml. of N/10 silver nitrate, 15 ml. of saturated solution of potassium chlorate and 5 ml. of dilute nitric acid. The mixture is then filtered and 50 ml. of the filtrate is titrated with N/10 ammonium thiocyanate using iron alum as indicator. Each millilitre of N/10 silver nitrate is equivalent to 0.0351 g. of $C_5H_{11}O_2N$.

Amyleni Hydras (*B.P. Add. III*). $C_5H_{12}O = 88.09$. Sp. gr., 0.814 to 0.817. Not less than 95% by volume distils between 100° and 104° . Other amyl alcohols, amylene and other organic impurities limited by mixing 1 part in 20 parts water with 0.25 ml. potassium permanganate solution, which should not be completely decolorised in 10 minutes; it should have no reducing action on ammoniacal silver nitrate on heating at 60° for 10 minutes. No cloudiness (limit of water) should be produced when mixed with an equal volume of light petroleum (b.p., 50° to 60°). Residue on evaporation and drying at 100° , not more than 0.01% *w/v*. Amyleni Hydras, *U.S.P. XII*, boils between 97° and 103° ; sp. gr. at 25° , 0.803 to 0.807, non-volatile residue not more than 0.25%. Limit tests are included for acidity, heavy metals, aldehyde, readily oxidisable substances and water.

ALCOHOL ISOPROPYLICUM

Alcohol Isopropylicum (B.P.C.). $(\text{CH}_3)_2\text{CHOH} = 60.06$. Boils between 80.5° and 81.5° and contains about 96% v/v (equivalent to about 94% w/w) of $\text{C}_3\text{H}_8\text{O}$; sp. gr., 0.810 to 0.812. It may be detected in methyl or ethyl alcohols by the production of a white or yellow precipitate on heating the liquid to be tested just to boiling with mercuric sulphate solution. It may be separated in solutions of ethyl and methyl alcohols by saturating with salt, when an upper layer of isopropyl alcohol separates.

Table for calculating the percentages of isopropyl alcohol in mixtures of isopropyl alcohol and water from their Zeiss refractometer readings at 20° to 25° , also a table of specific gravities at $20^\circ/20^\circ$ and $25^\circ/25^\circ$ of mixtures of isopropyl alcohol and water containing from 2 to 25% of the alcohol.—J. Batscha and S. Reznick, *J. Ass. off. agric. Chem., Wash.*, 1937, 107.

Detection of isopropyl alcohol.—*Amer. J. Pharm.*, 1931, 341; *Analyst*, 1931, 115.

Determination. A definite volume equivalent to about 15 ml. of 0.1% solution is heated with 0.4 g. of powdered potassium dichromate and 3 ml. of 4% sulphuric acid solution, shaken and allowed to stand for 24 hours. The mixture is then made alkaline with potassium hydroxide, diluted to 100 ml. and distilled. 60 ml. of distillate is heated with 40 ml. of N/1 potassium hydroxide and 25 ml. of N/10 iodine. After standing for 15 minutes, the liquid is acidified with a mixture of equal parts of hydrochloric acid and water and the excess of iodine titrated with N/10 sodium thiosulphate.—H. Kemal, *Z. anal. Chem.*, 1936, 107, 33.

Determination of acetone, isopropyl alcohol and alcohol in mixtures.—*See Adams and Nicholls, Analyst*, 1929, 2.

Isopropyl alcohol made in Great Britain is obtained by catalytic reduction of acetone. In perfumery, it is a good plan to blend with ethyl alcohol, the odour of isopropyl alcohol being too heavy and persistent when used alone. A table of solubilities of oils and synthetic perfumes in various spirits is given.—*Chem. & Drugg.*, 1/1927, 11.

Normal Butyl Alcohol. A British Standard Specification (B.S.S. No. 508—1933) has been issued by the British Standards Institution for Normal Butyl Alcohol (Butanol). The specification includes requirements regarding description, specific gravity, distillation-range, flash-point, residue on evaporation, acidity, aldehyde content and sampling, and the appendices describe the methods and apparatus to be used.

Dibutyl Phthalate. A British Standard Specification (B.S.S. No. 573—1934) has been issued by the British Standards Institution for Dibutyl Phthalate (di-normal-butyl ester of ortho-phthalic acid). The specification includes requirements regarding description, specific gravity, refractive index, water, ash, acidity, ester content and sampling, and the appendices describe the methods and apparatus to be used.

ALCOHOL METHYLICUM

Alcohol Methylicum (B.P.C.). $\text{CH}_3\text{OH} = 32.03$. Sp. gr. not higher than 0.799. Limit of ethyl alcohol is included by shaking with 10 volumes of 10% sodium hydroxide solution and 5 volumes of N/10 iodine solution when no turbidity or precipitate appears and no odour of iodoform develops when heated to from 60° to 70° for half an hour. On acidifying with acetic acid a mixture of 5 ml. of the alcohol, 2 ml. of normal sodium hydroxide and 0.2 ml. of 2% sodium nitroprusside solution, no violet colour is produced within one minute, limiting the acetone present. It may be estimated in alcoholic mixtures by matching with a series of control concentrations when oxidised with potassium permanganate and

phosphoric acid, decolorising after 10 minutes with oxalic and sulphuric acids and treating with decolorised magenta solution as described under Alcohol, previously making sure that the solution contains no formaldehyde.

A tentative method for the determination of methyl alcohol (0.5 to 5%) in the presence of ethyl alcohol is described in *Methods of Analysis (A.O.A.C., 1940, 611)*.

A British Standard Specification (B.S.S. No. 506—1933) has been issued by the British Standards Institution for Methyl Alcohol (Methanol). The specification includes requirements regarding description, specific gravity, distillation range, residue on evaporation, miscibility with water, acidity, aldehydes and ketones, sulphur and sulphur compounds, and sampling, and the appendices describe the methods and apparatus to be used.

Spiritus Methylatus Industrialis (B.P.). Sp. gr. not greater than 0.817. Residue at 100° not more than 0.01%.

Spiritus Methylatus Industrialis sine Acetone (B.P.C.). Sp. gr. not greater than 0.817. An acetone limit is specified: when to 5 volumes diluted with water to 10 volumes, 1 volume of 1% *o*-nitrobenzaldehyde in 50% alcohol and 1 volume of 15% sodium hydroxide solution are added the colour developed in 15 minutes should not be deeper than that produced by similarly treating 10 volumes of 0.025% *v/v* solution of acetone in 50% alcohol.

Acetone. Colorimetric method for determining and detecting in spirit, based on formation of indigo when sodium hydroxide is added to a mixture of *o*-nitrobenzaldehyde and acetone; can also be used for isopropyl alcohol after oxidation to acetone.—C. A. Adams, *Pharm. J.*, ii/1928, 604.

ALOE

Aloe (B.P.). Loss at 100° not more than 10%. Ash not more than 5%. Aloes, in powder, complies with the standard for the unground drug. On boiling with 100 parts of water until nearly dissolved, cooling, adding 1 part of diatomite and filtering, 5 ml. of the filtrate with 2 ml. of nitric acid gives a yellow-brown colour changing quickly to vivid green in the case of Cape aloes, Curaçao aloes gives a deep brownish red, in the case of the Socotrine variety a pale brownish yellow and with Zanzibar aloes a yellowish-brown colour is produced. In the *U.S.P. XII* the Socotrine and Curaçao varieties are official. Aloe, *U.S.P. XII*, should yield not more than 4% of ash, not more than 12% of moisture, and not less than 50% of water-soluble extractive determined by maceration and washing of the residue, evaporation and drying at 110°. Alcohol-insoluble substances, not more than 10%. Aloe, *P. Helv. V*, contains less than 12% of moisture and 1.5% of ash. Curaçao and Natal aloes are excluded and the product contains not less than 80% of non-resinous substances soluble in a mixture of 5 parts of methyl alcohol and 30 parts of chloroform.

Extract Content in Aloes. Average content water-soluble in Barbados aloes was 60%, and in Socotrine aloes 45% approx.

The proportion of aloes or other emodin-containing drug in a preparation may be determined by extraction with ether in the presence of acid and measuring the red colour that is produced when the ether extract is made strongly alkaline

with ammonia. Results are given for Socotrine, Cape and Curaçao aloes.—P. Valaer and G. E. Mallory, *Amer. J. Pharm.*, 1934, 81.

Differentiation of Aloes by Quinoline Hydrogen Peroxide Reagent. 30% hydrogen peroxide is shaken with an equal volume of quinoline (synthetic). Heat is evolved. At first the quinoline forms the top layer, but, after shaking, the lower. The quinoline is separated, dried with anhydrous sodium sulphate and filtered. Or in preference proceed more dilute. Finally dilute to 1% with more quinoline. A few mg. of finely powdered aloes are moistened with the reagent and warmed to 60° for 3 to 5 minutes. *A. chinensis* and *A. vulgaris* give purple, turning rose pink on acidifying with 1% sulphuric acid; *A. Perryi* brown with hydrogen peroxide, remaining so on acidifying; *A. ferox* and *A. spicata*, and others, green with hydrogen peroxide, changing to greenish yellow on adding acid.—E. J. Schorn, *Pharm. J.*, i/1930, 212; see also A. H. Ware, *ibid.*, 596.

Aloinum (B.P. Add. I). Ash not more than 0.5%. Water-insoluble matter not more than 1.5%, by shaking frequently for 2 hours and drying at 100°. The proportion of water-insoluble matter varies greatly with the temperature of the water, and samples giving less than 1.5% at 25° may yield as much as from 7 to 10% at 15°. The B.P. Add. I specifies a temperature of 25° to be maintained throughout this test and facilitates manipulation by filtration through a tared Gooch crucible. Aloinum, *U.S.P. XII*, leaves not more than 0.6% of ash; water-insoluble matter, not more than 1.5%, at 25°. A benzene extract should not impart a pink colour to an equal volume of 5% ammonia water.

Aloin may be determined in mixtures containing cascara, rhubarb, senna and other acid hydrolysable anthra-glucosides, resins and phenolphthalein by the official method of *Methods of Analysis (A.O.A.C., 1940)*. The substance dried for 1 hour at 110° and containing about 0.3 g. of aloin, mixed with 10 ml. of water and 5% sodium hydroxide, is diluted with 60 ml. and made acid with sulphuric acid as quickly as possible, diluted to 100 ml., shaken for 1 hour and filtered. 40 ml. of the filtrate with 10 ml. of sulphuric acid (10% w/w) is refluxed in a continuous extractor charged with chloroform. The aqueous solution is then saturated with salt and extracted with chloroform-alcohol (3:1), which is afterwards washed with 1 ml. of water with 1 g. sodium bicarbonate, filtered, evaporated and dried at 110° for 1 hour. The aloin can then be acetylated, and the aloin hexa-acetate extracted with chloroform and dried at 110°. Aloin hexa-acetate $\times 0.615 = \text{aloin}$.

ALUMINIUM

Alumen (B.P. Add. I). $\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O} = 474.4$ or $\text{NH}_4\text{Al}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O} = 453.3$. Contains not less than 99.5% of the appropriate pure substance. Ammonia alum leaves not more than 0.5% of residue when the filtrate from 1 g. in 100 ml. of boiling water and excess of dilute ammonia solution, is evaporated and ignited. Both alums are assayed gravimetrically by precipitation as aluminium hydroxide and ignition to Al_2O_3 . In the limit test for ammonium salts in potash alum, the limit allowed is equivalent to 0.1% of NH_3 , 0.01 g. being used for the test. Alumen, *U.S.P. XII*, is either ammonia alum or potash alum containing 99.5% of the pure alum. Limit of alkalis and alkaline earths, 0.5%.

Determination of Aluminium. Aluminium may be separated quantitatively from iron by precipitating the iron as sulphide in ammoniacal tartrate solution, and after removing the sulphides from the filtrate the aluminium is precipitated

as aluminium hydroxyquinolate by adding a 5% solution of 8-hydroxyquinoline sulphate to the ammoniacal solution. The mixture is heated to 90°, allowed to stand and the precipitate dried and weighed.—J. Haslam, *Analyst*, 1933, 270.

Aluminium can be determined by means of 8-hydroxyquinoline, *syn. oxine*, C_9H_7NOH . The slightly acid test solution containing about 0.02 g. of Al per 100 ml. is warmed to 50° to 60° and precipitated by the addition of the acetic 8-hydroxyquinoline solution in slight excess. The mineral acid is neutralised by the addition of 40 g. of ammonium acetate dissolved in water and the precipitate collected, washed with water, and dried at 110°. The residue, $Al(C_9H_6ON)_3$, contains 5.87% of Al. The precipitate may also be determined volumetrically as described for magnesium, after solution in concentrated hydrochloric acid and dilution with about 5 times the volume of water. Each millilitre of N/5 bromine is equivalent to 0.0004495 g. of Al.

The aluminium complex is obtained in the anhydrous form by drying at 98° and there is no particular advantage save that of speed in drying at higher temperatures.—R. C. Chirnside, C. F. Pritchard and H. P. Rooksby, *Analyst*, 1941, 402.

Small amounts of aluminium such as in plant ashes and fruit juices may be determined by precipitating the iron and aluminium as phosphates; the precipitate is dissolved in acid, the iron removed with cupferron and the aluminium precipitated with 8-hydroxyquinoline. Full practical details of the process are included.—L. Hart, *J. Ass. off. agric. Chem., Wash.*, 1932, 285.

Detection of Aluminium. Small amounts of aluminium can be detected and determined by means of Ammonium Aurine-tricarboxylate. *Syn. Aluminon*. $(C_6H_5-OH-COONH_4)_3$; C : $C_6H_5(COONH_4)$: O. The reagent is used as a 0.1% solution in water. To the solution to be tested, evaporated to about 5 ml. and acidified slightly with hydrochloric acid, is added 5 ml. of 25% w/v ammonium acetate solution and an equal volume of the reagent solution. In the presence of aluminium a red coloration or precipitate is produced on allowing to stand for 5 minutes. The colour is reduced in intensity if ammonium carbonate solution be added, but the similar lake formed by chromium is decomposed and interference from Cr ions thereby eliminated. The solution to be tested must be free from iron, and excess of alkaline earth metals must be avoided. The test may also be used quantitatively.

Alumen Exsiccatum (B.P.C.). $KAl(SO_4)_2 = 258.2$. Potash alum deprived of its water of crystallisation. Determined gravimetrically it contains not less than 99.5% of $KAl(SO_4)_2$, calculated on the dried substance. Loses at 200° not more than 10% of its weight. Alumen Exsiccatum of the *U.S.P. XII* may be either potash alum or ammonia alum heated at a temperature not exceeding 200° until aqueous vapours cease to be evolved. After drying at 200°, it contains not less than 96.5% of $AlNH_4(SO_4)_2$ or $AlK(SO_4)_2$. Assayed, after filtering out any matter insoluble in water, by precipitation as hydroxide and ignition to oxide. Yields not more than 2.5% of water-insoluble matter, dried at 100°; loss at 200°, not more than 10%.

Aluminii Sulphas (B.P.C.). $Al_2(SO_4)_3 \cdot 16H_2O = 630.4$. Assayed as for Alumen, it yields Al_2O_3 equivalent to not less than 99% of $Al_2(SO_4)_3 \cdot 16H_2O$. The substance of the *N.F. VII* is $Al_2(SO_4)_3 \cdot 18H_2O$, of which it should contain not less than 99.5% corresponding to a yield of 15.22% of aluminium oxide in the assay. Sulfate d'Aluminium, *Fr. Cx.* 1937, yields on incineration not less than 13% of Al_2O_3 .

Diatomite (B.P.C.). Purified kieselguhr from diatomaceous or infusorial earth. Moisture limit (on ignition), 10%. Ignited acid-soluble residue, not more than 1%. Terra Silicea Purificata, *U.S.P. XII*, is required to comply with the same limit for moisture and leaves an acid-soluble residue of 1.6%.

Kaolinum Ponderosum (*B.P. Add. VI*). Loss on ignition at a red heat, not more than 15%. Residue after ignition of the acid-soluble matter, not more than 1%. Kaolinum, *N.F. VII*, leaves not less than 85% of non-volatile residue on ignition. Ignited acid-soluble residue, not more than 2%.

Kaolinum Leve (*B.P. Add. VI*). Complies with the purity tests described under heavy kaolin, with a limit test for coarse particles, and limits for particles larger than 10μ diameter and for particles larger than 3μ diameter determined by taking the specific gravity of suspensions under stated conditions.

Talcum Purificatum (*B.P.C. Supp. IV*). Loss on ignition at red heat, not more than 2.5%. Water-soluble residue, not more than 0.2%. Ignited acid-soluble residue, not more than 0.5%. The *U.S.P. XII* substance is allowed 5% loss on ignition. Talc, *Fr. Cx.* 1937, has a density of 2.5 to 2.8.

Bentonitum (*U.S.P. XII*). Complies with a test for fineness of powder and loses 5 to 8% at 110° .

Sodium Silicate, Solution of. *Syn.* Soluble Glass, Water Glass. A viscid solution usually containing 10% of caustic soda and 20% of silica. *Liquor Natrii silicici, P.G. VI*, has a sp. gr. of 1.296 to 1.396. The solution will arrest the putrefaction of organic matter. In powder form, this silicate is used medicinally in France, but both it and silica would appear to be unsuitable chemicals for use *per os*.

Potassium Silicate, Solution of. *Syn.* Soluble Glass. This was the original preparation. It is less viscid than the sodium compound and is also used to impregnate bandages for treating fractures.

AMMONIUM

Ammonii Carbonas (*B.P.*). A variable mixture of bicarbonate ($\text{NH}_4\text{HCO}_3 = 79.05$) and carbamate ($\text{NH}_4\text{NH}_2\text{CO}_2 = 78.06$) Determined by dissolving in excess standard acid, boiling, cooling and back titrating with standard alkali using methyl red as indicator, it contains the equivalent of from 30 to 32.5% of NH_3 . Residue on volatilisation, not more than 0.025%. *Ammonii Carbonas, U.S.P. XII*, yields from 30 to 33% of NH_3 ; the titration is conducted without boiling and methyl orange is used as indicator. Ash, not more than 0.05%; the limit of sulphate allowed is two-fifths that of the *B.P.* substance, and a test for thiosulphate is included. Carbonate d'Ammonium, *Fr. Cx.* 1937, titrated to helianthin indicator, contains not less than 30% of NH_3 . Ammonium carbonicum, *P.G. VI*, consists of ammonium bicarbonate or a mixture of ammonium carbonate and ammonium bicarbonate containing from about 21 to 33% of NH_3 .

Liquor Ammonii Carbonatis (*B.P.C. Supp. II*). Determined as for *Ammonii Carbonas*, it contains the equivalent of 1.9 to 4.7% *w/v* of NH_3 .

Stability of Aqueous Solution. A 1 in 8 solution was found to be stable. —Corfield and Self, *Pharm. J.*, i/1926, 132.

Ammonii Bicarbonas (*B.P.*). $\text{NH}_4\text{HCO}_3 = 79.05$. Contains from 98% to the equivalent of 102% of NH_4HCO_3 , by adding

excess of acid, boiling, cooling and back titrating to methyl red. Residue on volatilisation, not more than 0.01%.

Liquor Ammoniaë Aromaticus (B.P. Add. V). Contains ammonia and ammonium carbonate equivalent to 2.1 to 2.4% *w/v* of NH_3 and 1.265 to 1.485% *w/v* of CO_2 . Determined by the assay described for Spiritus Ammoniaë Aromaticus.

Liquor Ammoniaë Fortis (B.P.). Has a specific gravity of 0.885 to 0.891 and contains not less than 31.5% and not more than 33.5% by weight of NH_3 . Methyl red is used as indicator for titration in excess normal sulphuric acid with normal sodium hydroxide. Residue on evaporation on a water-bath, not more than 0.01% *w/v*. Liquor Ammoniaë Fortis, *U.S.P. XII*, contains from 27 to 29% of NH_3 by weight. Assayed by direct titration after dilution with water with normal sulphuric acid to methyl red. Sp. gr., about 0.897 at 25°. Residue on evaporation and drying at 120°, not more than 0.06%. Ammoniaque Officinale, *Fr. Cx.* 1937, contains 20.18% NH_3 .

Liquor Ammoniaë Dilutus (B.P.). An aqueous solution containing not less than 9.5% and not more than 10.5% *w/w* of NH_3 . Residue on evaporation on a water-bath, not more than 0.005% *w/v*. Sp. gr., 0.958 to 0.9615. Liquor Ammoniaë Dilutus, *U.S.P. XII*, contains from 9 to 10% *w/v* of NH_3 , with sp. gr. about 0.957 at 25°. Residue on evaporation and drying at 120° not more than 0.02%. The dilute solution of the *Fr. Cx.* 1937 contains 10.09% NH_3 .

Spiritus Ammoniaë Aromaticus (B.P.). Contains ammonia and ammonium carbonate equivalent to 2.1 to 2.4% *w/v* of NH_3 , and 1.265 to 1.485% *w/v* of CO_2 . Total ammonia determined by addition of excess N/1 sulphuric acid, after boiling and cooling, back titration with N/1 sodium hydroxide to methyl red indicator. CO_2 determined by precipitation with barium chloride at 70°, and titration of the barium carbonate with excess N/1 hydrochloric acid and N/1 sodium hydroxide to methyl orange indicator.

Ammonii Sulphocyanidum. *Syn.* AMMONII RHODANIDUM (Thiocyanas). $\text{NH}_4\text{SCN}=76.1$. White crystals soluble in water and alcohol. Reagent in toxicology to separate arsenic, antimony, mercury, etc., and in silver titrations.

Hydrazine. *Syn.* DIAMIDE. $(\text{NH}_2)_2=32.05$. In the basic condition this body is not stable, but the sulphate, $(\text{NH}_2)_2\text{H}_2\text{SO}_4$, is a well-defined stable salt—white crystals soluble in hot water. It is a useful reducing agent, e.g., in making colloidal metal hydrosols. It has antiseptic properties, e.g., it will destroy fungi, etc.

PHOTOGRAPHIC USE. Caldwell discovered that the inclusion of the salts of hydrazine, or hydroxylamine, in the emulsion renders a plate practically proof against over-exposure or reversal. Plates or papers treated with the hydrazine salts may also be printed right out and toned like ordinary P.O.P., or partly printed and the operation completed by development.

AMYGDALA AMARA

Oleum Amygdalæ (B.P.). Sp. gr., 0.915 to 0.920; $n_{\text{D}40^\circ}$, 1.4624 to 1.4650; acid value, not more than 4.0; saponification

value, 188 to 196; iodine value, 95 to 100. After remaining at -10° for 3 hours, it remains clear and does not congeal until about -18° . Complies with the tests for absence of apricot-kernel and peach-kernel oils, cottonseed oil, sesame oil and arachis oil. *Oleum Amygdalæ Expressum, U.S.P. XII*, has a sp. gr. of 0.910 to 0.915 at 25° ; it should remain clear at -10° and not congeal until nearly -20° ; saponification value, 190 to 200; iodine value, 95 to 105. No solidifying-point of the fatty acids is specified.

Detection of Persic Oil in Almond Oil. In *Oils, Fats and Fatty Foods*, E. R. Bolton with reference to oils of the almond group states, "The oils of this group are remarkably similar in all their properties and their detection in the presence of one another may be taken to be almost a practical impossibility" and "of the many tests which have been described for the purpose of achieving the impossible, probably Bieber's test is the least unsatisfactory." Many modifications of the test have been published. The following modification of the *B.P.* nitric acid test will detect the presence of 5% of apricot-kernel oil. Four drops of oil are shaken with 4 drops of chloroform, and two drops of fuming nitric acid are then added separately down the sides of the tube, with 10 seconds interval between each drop. Apricot-kernel oil gives an immediate blood-red colour changing to brownish-red; peach-kernel oil gives a red colour within one minute; almond oil, a light brown colour within two minutes. Fresh mixtures of almond oil with 5% of apricot oil give a brilliant red within 5 minutes; old mixtures require 15 minutes to develop the full colour. Peach-kernel oil is not a commercial article and is probably never used as an adulterant of almond oil.—*Z. anal. Chem.*, 1933, 94, 184.

The **Titre Test** is the determination of the solidifying-point of the fatty acids. In the *U.S.P. XI*, under tests for identity and purity, almond oil was required to have a solidifying-point of the fatty acids: not less than 9° and not more than 12° , exact details of the method of preparing the fatty acids and the apparatus to be used being specified. This requirement excludes almond oils which are undoubtedly genuine, such oils whilst they show a titre test at about 12° or under give a much more definite figure at about 6° . In neither case is the rise in temperature so definite as with some other oils such as palm kernel or coconut. The two figures are probably due to the formation of eutectic compounds in the mixture of fatty acids which are present.

Elaidin Test. A modification of this test in which the percentage of the elaidin crystallised from acetone, its melting-point, iodine value and saponification value are taken may be applied to almond oil, which yields 26.8 to 29.3% of elaidin with a melting-point of 38.5° to 39° ; iodine value, 78.5 to 79.5 and saponification value, 191.5 to 191.3. These figures differ distinctly from those of ground-nut oil and very widely from those of cottonseed oil. For further details of the test see elaidin test under olive oil (p. 293, this volume) and H. N. Griffiths and T. P. Hilditch, *Analyst*, 1934, 312.

Oleum Persicæ (B.P.C.). Sp. gr., 0.917 to 0.921; n_{D40}^{20} , 1.464 to 1.465; acid value, not more than 8; saponification value, 189 to 193; iodine value, 100 to 110. *Oleum Persicæ, U.S.P. XII*, must be labelled to indicate whether derived from apricot kernels or peach kernels. Sp. gr. at 25° , 0.910 to 0.918. Tests for paraffin oil, cottonseed oil and sesame oil are included. Saponification value, 185 to 195. Iodine value, 90 to 108. Does not become turbid at 15° .

Perilla Oil. The oil obtained by expression or extraction from the seeds of *Perilla ocymoides*, Fam. Labiatae, growing in China, India and Japan. The seeds contain from 36 to 43% oil. *B.S.S. No. 654—1936*, includes the following:—Sp. gr., 0.932 to 0.936; n_{D20}^{20} , 1.481 to 1.484; iodine value, not below 193; saponification value, not below 189; acid value, not above 6; unsaponifiable matter, not more than 1.5.

Oleum Amygdalæ Amaræ (B.P.C.). Contains not less than 85% *w/w* of C_6H_5CHO and the equivalent of 2 to 4% *w/w* of HCN . Sp. gr., 1.055 to 1.065. Refractive index at 20° , 1.534 to 1.542. Assayed by the *B.P.C.* method for benzaldehyde, in which standard hydroxylamine hydrochloride is added to the oil, and titrated with *N/2* alcoholic potassium hydroxide, using methyl orange as indicator, allowance being made for the free benzoic acid present. Hydrocyanic acid is determined by titration of the alcoholic solution, made slightly ammoniacal, with standard silver nitrate to a permanent opalescence with potassium iodide. A benzoic acid limit, equivalent to approximately 2%, is included and the oil should comply with the *B.P.C.* limit test for chlorinated compounds in Benzaldehydum. Nitrobenzene is tested for by reduction of an alcoholic solution with zinc powder and acetic acid, the presence of any aniline formed being detected by the odour of phenyl isocyanide on warming the mixture with chloroform and excess of sodium hydroxide. The *U.S.P. XII* requires a sp. gr. of 1.038 to 1.06 at 25° and the same strength of hydrocyanic acid; a lower standard of 80% of benzaldehyde is required, and is determined by addition to a neutralised solution of hydroxylamine hydrochloride in alcohol (60%) and titration with *N/2* alcoholic potash using bromophenol blue indicator. Hydrocyanic acid is titrated with silver nitrate, using potassium chromate as indicator, in the presence of magnesium sulphate and sodium hydroxide. Nitrobenzene is tested for by reduction to aniline as in the *B.P.C.* n_{D20° , 1.5428 to 1.5439.

The Food and Drug Administration of the U.S. Dept. of Agriculture defined oil of bitter almonds, commercial, for food purposes, as the volatile oil from the seed of the bitter almond (*Amygdalus communis* L.), the apricot (*Prunus armeniaca* L.), or the peach (*Amygdalus persica* L.).—*S.R.A., F.D. No. 2, Rev. 5, Nov. 1936.*

In America, benzaldehyde is largely substituted for oil of bitter almonds. Frequently hydrocyanic acid in sufficient quantity is added to meet the requirements of the trade or the *U.S.P.* Adulteration with benzaldehyde can only be detected if the synthetic benzaldehyde contains chlorine. For method of assay see Salamon, *Perfum. essent. Oil Rec.*, 1917, 41. Much of the synthetic benzaldehyde is now produced by processes which do not involve the use of chlorine and is therefore free from that impurity. Benzaldehyde is considered to be inferior as a flavouring agent to natural oil of bitter almonds.

Small quantities of chlorine may be detected by a modification of the burning method; the combustion vapours being passed over silver gauze and the deposited silver chloride estimated.—Daudney, *Analyst*, 1935, 29.

Oleum Amygdalæ Amaræ sine Acido Hydrocyanico (B.P.C.). Estimated by the method for Benzaldehydum, should contain 95% by weight of C_6H_5CHO . Sp. gr., 1.048 to 1.052. Refractive index at 20° , 1.540 to 1.545. Limits for benzoic acid and nitrobenzene as for Oleum Amygdalæ Amaræ, and should not contain any hydrocyanic acid as shown by addition of sodium hydroxide and ferrous sulphate and acidification with hydrochloric acid, when no greenish blue colour or precipitate is produced. Complies with the test for chlorinated compounds in Benzaldehydum.

Oleum Amygdalæ Volatile Purificatum (*B.P. Add. II*). Contains not less than 95% of benzaldehyde, C_6H_5CHO . Soluble in 2 parts of alcohol (70%). Sp. gr. ($15.5^\circ/15.5^\circ$), 1.048 to 1.052. n_{D20° , 1.542 to 1.546. Tests for absence of hydrocyanic acid and for limit of chlorinated compounds are included. Determined by the *B.P.* process for aldehydes in volatile oils, using 0.5 g. of oil and 12 ml. of hydroxylamine hydrochloride reagent in alcohol (60%).

Benzaldehydum (*B.P.C.*). $C_7H_6O=106.0$. By titration of the acid liberated by interaction with N/2 hydroxylamine hydrochloride with N/2 alcoholic potassium hydroxide to methyl orange, and allowance for the free benzoic acid present, it contains not less than 90% of C_7H_6O . Sp. gr., 1.049 to 1.053; n_{D20° , 1.554 to 1.556. Complies with tests for hydrocyanic acid, nitrobenzene, chlorinated compounds and a limit of benzoic acid. The *N.F. VII* assays by a similar method and requires it to contain not less than 98% of C_7H_6O .

AMYLUM

Amylum (*B.P. Add. I*). Starch obtained from the grains of maize, *Zea Mays* Linn., or of rice, *Oryza sativa* Linn. Loss at 100° , not more than 14%. Ash, not more than 0.5%. Amylum, *U.S.P. XII*, is derived from the grain of *Zea Mays* Linné only. Moisture not more than 14% and ash not more than 0.5%. A trituration with 10 parts of water should be neutral to litmus paper. Complies with a limit test for iron—0.5 g. with 10 ml. distilled water, on the addition of 0.5 ml. of hydrochloric acid and 3 drops of potassium ferrocyanide solution, does not become blue within one minute.

For a gravimetric method for the determination of starch in substances generally, see J. J. Chinay, F. W. Edwards and H. R. Nanji, *Analyst*, 1934, 673.

Details with diagrams, of the microscopical structure of ten common commercial starches.—T. E. Wallis, *Pharm. J.*, ii/1933, 396.

Volumetric Method of Determination. Gelatinise the starch by heating with aqueous potassium hydroxide. Cool, dilute and adjust to a definite volume with water. Put a quantity in a centrifuge tube and make faintly acid to phenolphthalein with acetic acid. Add definite amounts of N/10 iodine, 2N potassium acetate and adjust with water to a definite volume. Centrifuge and pipette off part of the clear liquid and titrate with N/10 thiosulphate, using starch as indicator. Then titrate precipitate and liquid remaining in the tube. In calculating the result a factor is necessary because the composition of starch iodide varies with the excess of iodine left in contact with the precipitate. The factor is obtained from a specially prepared chart.—W. Whale, *Analyst*, 1938, 328–421.

Maranta (*B.P.C.*). Arrowroot is prepared from the rhizomes of *Maranta arundinacea* Linn. Loss at 100° , not more than 20%. Ash, not more than 0.3%.

The government of St. Vincent have defined arrowroot as the separated and purified starch of *Maranta arundinacea*. The starch similar to sweet potato starch with which arrowroot has been adulterated is obtained from "marble arrowroot," the bulbs of *Myrosma canifolia*. It is occasionally mixed with arrowroot by the growers.—P. H. Jones *Food*, 1934, 225.

ANETHUM

(with ANISUM and ANTHEMIS)

Anethum (B.P.). Contains not more than 2% of foreign organic matter, and not more than 11% of ash.

The Food and Drug Administration of the U.S. Dept. of Agriculture defined dill seed as the dried fruit of *Anethum graveolens* L. Required to contain not more than 10% total ash, nor more than 3% ash insoluble in hydrochloric acid. —S.R.A., F.D. No. 2, Rev. 5, Nov. 1936.

Oleum Anethi (B.P.). Carvone content not less than 43% and not more than 63% by weight. Determined by the method of the B.P.: About 1.5 g. is accurately weighed into a stoppered tube, about 150 mm. long and 25 mm. diameter, 10 ml. of a hydroxylamine reagent (70 g. of hydrochloride in 900 ml. of 90% alcohol, 4 ml. of dimethyl yellow solution, sufficient N/1 alcoholic potash to produce the full yellow colour of the indicator, and alcohol to 1000 ml.) added, and placed in a water-bath at 75° to 80°; neutralisation of the liberated acid with N/1 alcoholic potash and further heating in the water-bath is continued alternately until the full yellow colour is permanent for 5 minutes heating (about 35 minutes total heating). A duplicate determination is performed using the first titration with an additional 0.5 ml. of N/1 alcoholic potash as colour standard for the titration end-point; from the second titration each millilitre of N/1 alcoholic potash is equivalent to 0.1513 (0.1501 × 1.008) g. of carvone. The Pharmacopœia requires the oil to be soluble in an equal volume of alcohol (90%, sp. gr. 0.8334 to 0.8340) and in 10 volumes of alcohol (80%, sp. gr. 0.8634 to 0.8640). Sp. gr., 0.900 to 0.915. Optical rotation, + 70° to + 80°. Refractive index at 20°, 1.481 to 1.492.

Few oils contain more than 60% of carvone.—*Perfum. essent. Oil Rec.*, ii/1932, 199.

A method of applying the hydroxylamine reaction in the determination of carvone in dill oil.—Bennett and Cocking, *Quart. J. Pharm.*, 1931, 580.

Anisum (B.P.C.). The dried ripe fruits of *Pimpinella Anisum* Linn. Contains not more than 2% of other seeds and fruits and not more than 1% of foreign organic matter; acid-insoluble ash, not more than 1.5%. Anisum, N.F. VII, contains not more than 3% of foreign organic matter and not less than 1.75% of volatile oil. Acid-insoluble ash, not more than 1.5%. No mouse-like odour, due to the presence of *Conium maculatum* fruit, develops when 1 g. is heated with 10 ml. of potassium hydroxide solution. Fructus Anisi, P.G. VI, yields not less than 1.5% of volatile oil.

The Food and Drug Administration of the U.S. Dept. of Agriculture defined anise, aniseed, as the dried fruit of *Pimpinella anisum* L.; not more than 9% total ash or more than 1.5% ash insoluble in hydrochloric acid. Star aniseed was defined as the dried fruit of *Illicium verum* Hook., and contained not more than 5% total ash.—S.R.A., F.D., No. 2, Rev. 5, Nov. 1936.

Oleum Anisi (B.P.). Distilled from Anisum or from the dried fruits of the star anise, *Illicium verum* Hook. f. Should be soluble in 3 volumes of alcohol (90%, sp. gr. 0.8334 to 0.8340), showing not more than a slight opalescence. Sp. gr. (20°/15.5°), 0.980 to 0.994. Optical rotation, - 2° to + 1°. Refractive index at 20°,

1.553 to 1.560. Freezing-point, not below 15°. M.p., not below 17°. Should comply with a limit for lead. Oleum Anisi, *U.S.P. XII*, may be distilled from the dried ripe fruits of either *Pimpinella Anisum* Linné or *Illicium verum* Hooker filius. Soluble with not more than a faint opalescence in 3 volumes of 90% alcohol, the similar solution of the recently distilled oil being neutral to litmus paper and developing no blue or brownish colour with 1 drop of ferric chloride solution. Sp. gr. at 25°, 0.978 to 0.988. Refractive index at 20°, 1.5530 to 1.5600. α_D at 20°, +1° to -2°.

The lead often contained in this oil is derived from the leaden containers in which most of the oil is imported.

Toxicity of Old Sample of Aniseed Oil. Old oil which can no longer be made to crystallise on cooling shown to be toxic on external application to animals.—Samdahl, *Quart. J. Pharm.*, 1932, 588.

Anthemis (B.P.C.). Dried double or semi-double flower heads of *Anthemis nobilis* Linn. Foreign organic matter, not more than 2%, and acid-insoluble ash, not more than 1%.

Oleum Anthemidis (B.P.C.). Soluble in 6 volumes of alcohol (70%, sp. gr., 0.8896 to 0.8901), forming a clear solution. Sp. gr., 0.905 to 0.915. Refractive index at 20°, 1.442 to 1.448. Acid value, 1.5 to 14.0. Saponification value, 260 to 296.

Matricaria (B.P.C.). Dried flowerheads of *Matricaria chamomilla* Linn. Contains not more than 8% of its stems and other foreign organic matter. Yields not more than 4% of acid-insoluble ash. The *N.F. VII* allows 10% of stems and not more than 2% of other foreign organic matter in *Matricaria*; the acid-insoluble ash limit is 4%.

ANTIMONIUM

Antimonii Oxidum (B.P.C. Supp. IV). $Sb_2O_3 = 291.5$. As assayed by titration with N/10 iodine of a solution in dilute hydrochloric acid, with the addition of sodium potassium tartrate and a slight excess of sodium bicarbonate, it contains not less than 95% of Sb_2O_3 . Completely soluble when boiled with excess of potassium acid tartrate solution.

Acid-Resistance Test for Enamel. The solubility of enamel on enamelled ironware is dependent on the strength of the solutions of organic acids with which it comes in contact. It should be free from antimony, lead, arsenic, and other deleterious or poisonous ingredients. Acid-resistance may be controlled by the following test:—"Enamelware vessels will be considered to be sufficiently resistant to acid if, when filled as full as is convenient with a boiling 0.5% solution of citric acid in water and allowed to stand for 24 hours without being heated or artificially cooled, the amount of ash yielded on ignition of the residue obtained when a definite proportion of the solution is evaporated does not exceed 1.0 mg. per sq. cm. of surface exposed to the action of the acid, and if on repetition of the treatment with a fresh similar volume of the boiling acid solution not more than a further 0.5 mg. per sq. cm. is obtained." Antimony should be absent from the constituents used in making the enamels. Acid extracts may contain appreciable quantities of borax and significant amounts of fluorine and the exclusion of constituents containing these elements is desirable.—J. H. Coste and D. C. Garratt, *Analyst*, 1935, 215.

The determination of antimony compounds extracted from enamelware by citric acid solution.—R. H. Burns, *Analyst*, 1935, 220.

Antimony Detection in Biological Liquids. The reagent used is 1 g. of phenazone and 2 g. of potassium iodide in 30 ml. of water. Biological fluids are evaporated and ignited, the residue dissolved in hydrochloric acid and the

reagent added; 0.025 mg. of antimony can be detected.—*J. chem. Soc. Abstr.*, ii/1923, 587.

Determination. The antimony solution, mixed with twice its volume of reagent (100 g. sodium thiosulphate, 200 ml. of water and 150 ml. of sulphuric acid) is heated on a water-bath for 30 minutes. It is filtered through a sintered glass crucible, washed with 25% sulphuric acid, and the precipitate, antimony, dissolved by passing an excess of N/10 iodine containing 1 ml. of 10% tartaric acid through the crucible. A volume of standard arsenite solution, equivalent to the volume of iodine solution used is added with sodium bicarbonate and the excess arsenite titrated with N/10 iodine. The reaction can also be used as a nephelometric means of determination. To determine antimony in the presence of arsenic, the total arsenic is determined, the combined arsenic and antimony precipitated by the above process is determined and also the arsenic remaining in the filtrate, because only about one-third of the arsenic present is precipitated.—L. Fauchon and L. Vignoli, *J. Pharm. Chim., Paris*, 1937, 25, 541.

Antimonii et Potassii Tartras (B.P.). $C_4H_4O_7SbK, \frac{1}{2}H_2O = 333.9$. Should contain not less than 99% of the pure substance. Assayed by titration of the aqueous solution with N/10 iodine, with the addition of sodium bicarbonate and using starch mucilage as indicator. It is required to comply with a test for alkalinity or acidity by which 1 g. requires for neutralisation to the green colour of bromocresol green indicative of pH 4.5 not more than 2.0 ml. of either N/100 sulphuric acid or N/100 sodium hydroxide. The U.S.P. XII substance is required to be of the same purity; the usual limit test for arsenic, is not used for this substance. 0.1 g. of the salt is dissolved in 5 ml. of hydrochloric acid and 10 ml. of a solution containing 20 g. of stannous chloride in 30 ml. of hydrochloric acid added. After mixing, the tube is allowed to stand 30 minutes and the colour developed is then compared with that of a similar tube containing none of the antimony compound but to which has been added 0.02 mg. of arsenic trioxide. Viewed downward over a white surface the colour of the mixture is not deeper than that of the standard tube. *Tartarus stibiatus, P.G. VI*, contains not less than 99.5% of $C_4H_4O_7SbK, \frac{1}{2}H_2O$.

Antimonii et Sodii Tartras (B.P.). $C_4H_4O_7SbNa = 308.8$. Loses not more than 5% of its weight at 100° and then contains not less than 96% of $C_4H_4O_7SbNa$. The same limit of acidity or alkalinity as for the potassium compound is included and the same method of assay is used. No limit of arsenic is specified.

Stibophenum (B.P. Add. III). $C_{12}H_4O_{16}S_4SbNa_5, 7H_2O = 895.1$. Contains 15.6 to 16.0% of trivalent antimony and 16.5 to 16.9% of sulphur, calculated on the dried substance. Complies with tests for limit of calcium, absence of chloride, and of sulphate. Loss on drying at 140° to 150° in a vacuum, 14 to 16%. Trivalent antimony determined by addition of excess iodine solution to a solution in dilute acetic acid and back titration with standard sodium thiosulphate. Sulphur determined by ignition with potassium hydroxide and potassium nitrate and precipitation as sulphate.

ORGANIC ANTIMONY COMPOUNDS.

Biological Tests. These substances are tested biologically for toxicity and therapeutic potency in the same way as are the organic arsenicals.

Toxicity Tests. Toxicity tests are usually carried out on mice by injection into the tail vein, and the dose is determined which kills 50% of mice.

Therapeutic Potency. Tests for therapeutic potency may be made as described for nearsphenamine, but a better method has been described by Gray, Trevan, Bainbridge, and Attwood (*Proc. roy. Soc., Ser. B.*, 1931, 108, 54). Mice are infected with trypanosomes, but a smaller dose of infected material is given than for the nearsphenamine test. Instead of leaving the mice for two days during which the infection develops unchecked, the agent to be examined is injected at once, ten infected mice each receiving the same dose, and ten other mice each receiving a dose of the substance used as a standard of comparison. No daily examination of the blood of the mice is made, but the number of days each mouse survives is observed. The average period of survival of mice injected with the substance being tested is determined, and also the average period of survival of mice injected with the standard. Should these average survival times not be the same, the experiment is repeated until a dose of the sample is found which gives an average survival time about equal to that given by the chosen dose of the standard.

AQUA DESTILLATA

Aqua Destillata (B.P.). Residue on evaporation on a water-bath, not more than 0.001%. After 5 minutes 10 ml. portions show no reactions for chlorides and sulphates with silver nitrate and barium chloride solutions respectively. 100 ml. (testing for lead, copper and iron) should remain clear and colourless with 1 drop of sodium sulphide solution. Limit of ammonia (1 part NH_3 in 2.5 millions) by Nesslerising 50 ml. Oxidisable matter is limited by boiling 100 ml. for 10 minutes with permanganate, when the colour should not be completely destroyed. The *U.S.P. XII* water should have a pH of 5.0 to 7.0, as shown by the reactions to methyl red and bromothymol blue pH indicators: residue on evaporation and drying at 100° , not more than 0.001%. Tests for sulphate, chloride, calcium, heavy metals, ammonia and carbon dioxide are included.

Aqua Sterilisata (B.P. Add. I). The distillate (excluding the first portion) collected in a sterilised neutral glass container, obtained from potable water using a glass still, or one in which the distillate is not in contact with copper, cleansed immediately before use. Should be sterilised by heating in an autoclave, closing the container with a plug of sterile non-absorbent cotton wool wrapped in gauze, by fusion of the glass or other effective method. When stored in a container closed with cotton wool it may be used within one month only. On using part of the contents of a container the remainder may be stored after sterilisation. For use within 24 hours it may be prepared by boiling for 30 minutes in a plugged container. Complies with tests for sulphates, chlorides, for lead, copper and iron, ammonia, oxidisable matter and residue. Aqua Destillata Sterilisata, *U.S.P. XII*, complies with the tests for distilled water and the sterility test for liquids of the *U.S.P. XII*. It is not to be used for parenteral administration or in preparations used parenterally.

Distilled water for parenteral injections. Reports of the production of rise of temperature and rigors following the intravenous injection of large volumes of perfusion fluids are not

uncommon. It was first investigated by Wechselmann (1911) in respect of Salvarsan-saline injections. Bacteria can multiply in sterile distilled water, though they ultimately die. It is unlikely that pyrogenic symptoms arise from the presence of protein from dead bacteria. Seibert (*Amer. J. Physiol.*, 1923, 67; *ibid.*, 1925, 71) in a series of papers investigated several suggested causal factors and eliminated the following: alkalinity from the glass vessels, the extractive matter from ordinary corks, gases such as CO₂, O₂, H₂S, NO₂, hæmolysis of the blood due to hypotonicity, the pH of the solutions. She concluded that the causal factor must be of bacterial origin, and showed that all waters, tap or distilled, were not pyrogenic. The causative organisms were certain water-borne bacteria producing an excretory product, *pyrogen*, which was capable of passing the usual bacterial filters and was very thermostable; long and drastic heating such as refluxing for 7 hours was necessary to destroy it. Unless a water-still is provided with a splash head, pyrogen may be carried over to give a pyrogenic distillate. It is also possible for the condenser to become contaminated with living pyrogenic organisms and for the distillate to contain them. Such a distillate would develop its maximum pyrogenic properties in 4 days. *Hence it is essential that distillation should be followed by immediate sterilisation.* This will prevent the development of pyrogen in the distillate.

The British Pharmacopœia (*Add. I*) specifies immediate sterilisation of fresh distillate in the preparation of sterilised water and places no limit of storage on such water if stored in hermetically sealed containers.

It is possible that pyrogenic symptoms may also be due to other causes. Thus Hyman and Hirschfeld (*J. Amer. med. Ass.*, ii/1933, 305) and Hirschfeld (*Arch. int. Med.*, 1931, 47, 259) claim that an excessive speed in injecting can cause a syndrome which they term "speed shock."

Aqua Pro Iniectione (*U.S.P. XII*), is water which has been distilled and sterilised within 24 hours and is for parenteral use. It is stored in sealed or other suitable containers, so that it is free and remains free from pyrogens. Complies with the physical and chemical standards of Aqua Destillata, *U.S.P. XII*, and with the sterility test for liquids. A special pyrogen test is included; 10 ml. per kg. body weight is injected intravenously into healthy rabbits and hourly observations of the body temperature are made. Not less than 5 animals are used and the test is positive if 3 animals out of 5 show an individual rise in temperature of 0.6°C. or more above the normal previously established for the animals. If 2 animals out of 5 show a positive response, a further 5 animals must be tested and the test is positive if 2 out of the second group show a positive response. Strictly controlled conditions must be observed in carrying out the test.

The pyrogen found in infusion fluids is of a particulate nature, of a larger order of magnitude than 50 millimicrons but smaller than one micron. There are now available two processes of filtration which effectively remove the reactive agent from infusion fluids. One is ultrafiltration which, however, is

too slow and too difficult for clinical use, and the other s adsorptive filtration through two layers of Seitz serum No. 3 filter pads (compressed asbestos), which is easy to manipulate and should be feasible for use in hospitals and commercial houses. The new process may be summarised as follows: Distillation → addition of chemicals → adsorptive filtration → sterilisation.—Co Tui *et al.*, *J. Amer. med. Ass.*, ii/1937, 250.

Pyrogen-free water may be prepared in an emergency by shaking the water for fifteen minutes with an adsorption agent and then passing through a sintered glass filter. Activated powdered charcoal, 1 g. per l. of water, was highly effective in removing pyrogens.—J. C. Lees and G. A. Levvy, *Brit. med. J.*, i/1940, 430.

Pyrogens may sometimes occur in medicaments themselves. This fact is largely overlooked, and solutions may be contaminated by the solute after very careful preparation of the distilled water. Substances such as dextrose are particularly liable to contamination and require special precautions. In the preparation of non-pyrogenic solutions, distillation was used to obtain non-pyrogenic water, and the method of Lees and Levvy, of adsorbing pyrogens on activated charcoal, to deal with pyrogens in the medicaments. Biological tests confirmed the efficiency of the method.—J. P. Todd, *Pharm. J.*, i/1941, 258.

ARECA

Areca (B.P.C.). The dried ripe seeds of *Areca Catechu* Linn. The *N.F. VII* requires Areca to contain not more than 2% of adhering pericarp, not more than 1% of foreign matter, not more than 2.5% of ash, and not less than 0.35% of ether-soluble alkaloids calculated as arecoline. The drug in powder is assayed by shaking with ether in the presence of ammonia followed by addition of anhydrous sodium sulphate and further shaking. The ether solution is then decanted, shaken with talc and allowed to stand. An aliquot portion of the ether solution is decanted, extracted with N/50 sulphuric acid and washed with water. The combined acid and washings are then titrated against N/50 sodium hydroxide using methyl red as indicator. *Semen Arecæ, P.G. VI*, assayed by the process prescribed, contains not less than 0.4% of alkaloid, calculated as arecoline. *Semen Arecæ, P. Helv. V*, contains not less than 0.4% of alkaloids.

P.G. VI Assay. Digest 8 g. of powdered areca with 80 g. of ether, and 4 g. of ammonia solution (9.94 to 10%) and shake the mixture for 10 minutes. After the addition of 10 g. of anhydrous sodium sulphate shake for 5 minutes, pour off the ethereal solution immediately from the mixture, add 0.5 g. of talc, shake for 3 minutes, add 2.5 g. of water and shake. Pour off 50 g. of the dry ethereal solution into a small flask and distil off two-thirds of the ether. Run the cold residue into a separating funnel, rinsing the flask three times with 5 ml. of N/10 hydrochloric acid and 5 ml. of water. After shaking for 3 minutes, let the hydrochloric acid become completely clear, run off into a flask and then repeat the shaking three times in the same manner with 5 ml. of water. Now add to the hydrochloric acid 2 drops of methyl red solution and titrate with N/10 potassium hydroxide until the colour changes. Not more than 3.71 ml. of N/10 potassium hydroxide should be used, since 1.29 ml. of N/10 hydrochloric acid is equivalent to 0.4% of alkaloid. (1 ml. N/10 HCl = 0.015511 g. of alkaloid).

P. Helv. V Assay. The powdered drug is defatted with light petroleum and then extracted with ether; the dried ethereal extract is extracted repeatedly with standard dilute hydrochloric acid and the excess of acid titrated with N/10 sodium hydroxide using methyl red as indicator. Each millilitre of N/10 HCl is equivalent to 0.0155 g. of alkaloid.

Arecolinæ Hydrobromidum (N.F. VII). $C_{12}H_{19}O_2N.HBr = 236.1$ Loss over sulphuric acid for 24 hours, not more than 1%. Ash, not more than 0.5%.

Tests for free acid, other alkaloids and sulphates are included. Bromhydrate d'Arécoline, *Fr. Cx.* 1937, melts at 170°, and the chloroplatinate at 176°, and complies with an ash limit and a test for foreign alkaloids.

Arecolinum hydrobromicum, *P. Helv. V.*, contains 99.4% of $C_8H_9O_2N.HBr$ and is assayed by titration with N/10 sodium hydroxide. M.p., 169° to 171°.

Methods for the determination of arecoline and other constituents of areca nut.—P. Bourcet, *Brit. chem. Abstr. B.*, 1933, 1035.

ARGENTUM

Argenti Nitras (B.P.). $AgNO_3=169.9$. Contains not less than 99.8% of the pure salt. Titrated with N/10 ammonium thiocyanate, using ferric ammonium sulphate as indicator. A limit of copper, bismuth and lead is included. The *U.S.P. XII* also requires a purity of 99.8%.

Detection of Silver. The presence of silver may be shown by means of *p*-dimethylaminobenzylidenerhodanine (*syn. p*-dimethylaminobenzalrhodanine). The reagent is used as a 0.03% solution in acetone. To 20 ml. of the approximately neutral test solution add 2 ml. of N/1 nitric acid and 0.5 ml. of the reagent. The presence of silver is denoted by the production of a reddish coloration. In the presence of mercuric compounds 0.5 ml. of N/1 hydrochloric acid must be added after the reagent. In the presence of gold, platinum or palladium 0.5 ml. of 10% potassium cyanide solution must be added to the test solution, followed by the reagent and then the nitric acid. If copper is present, it must be oxidised to the cupric state, no coloration being then produced in acid solution. Cuprous ions give a violet precipitate or coloration.

Argenti Nitras Induratus (U.S.P. XII), by titration of the water-soluble portion with ammonium thiocyanate, contains 94.5% of $AgNO_3$.

Liquor Argenti Nitratiss Ammoniacalis (N.F. VII). Contains 28.5 to 30.5% w/w of Ag and from 9.0 to 9.7% w/w of NH_3 .

Argentoproteinum (B.P. Add. I). Contains from 7.5 to 8.5% of Ag. Assayed by titration with tenth normal ammonium thiocyanate of the slowly incinerated residue heated with nitric acid till no more coloured fumes are evolved, and diluted. The filtrate from 1 g. shaken with 10 ml. of alcohol (90%) should produce no turbidity on the addition of 2 ml. of dilute hydrochloric acid. Argentum Proteinicum Forte, *U.S.P. XII*, contains from 7.5 to 8.5% of silver. The following test for distinction from Mild Protein Silver is included:—7 g. of ammonium sulphate is added to 1 g. in 10 ml. of water and stirred for 30 minutes. After filtration, to the clear liquid is added 25 ml. of 1% acacia solution. The turbidity produced on adding 2 ml. of nitric acid and 2 ml. of diluted hydrochloric acid and acacia solution to 50 ml. is *not* greater than that produced by similarly treating 10 ml. of water with 1.6 ml. of N/100 silver nitrate.

Argentum colloidal, *P.G. VI*, contains not less than 70% of silver and Argentum proteinicum contains at least 8%. Argentum colloidal, *P. Helv. V.*, contains not less than 70% of silver, and Argentum proteinicum, *P. Helv. V.*, from 8.0 to 8.3%. The silver is converted by careful ignition and subsequent treatment with nitric acid into silver nitrate which is then titrated with ammonium thiocyanate using iron alum as indicator. Argent colloidal par voie chimique, *Fr. Cx.* 1937, assayed by oxidation with sulphuric acid and potassium permanganate and titration with N/10 ammonium thiocyanate, contains not less than 70% Ag.

Argenti Proteinis Mite (B.P.C.). Assayed by the method for the stronger proteinate, contains from 19.0 to 25.0% of Ag.

It complies also with a limit test for silver salts. Argentoproteinicum Mite, *U.S.P. XII*, contains 19 to 23% of Ag. The turbidity produced in the same test for distinction used for the strong preparation of the *U.S.P. XII*, should be *no* greater than the control test. (*Note*.—Obviously, one of these two tests for distinction is wrongly stated in the *U.S.P. XII*.)

Official methods for the determination of total silver and ionisable silver compounds in silver proteinates are described in *Methods of Analysis* (*A.O.A.C.*, 1940, 624).

Preparation of Colloidal Silver and Silver Proteinate. Add slowly with constant stirring 1 kg. of casein to a hot solution of 0.5 kg. sodium hydroxide solution (40° Bé, sp. gr. 1.383) and 2 l. of water. Dilute with half its volume of water, cool and filter. Acidify with nitric acid diluted with 2 parts of water and knead the precipitated protalbic acid with water to remove nitric acid, adding the washings to the mother-liquor which contains lysalbic acid. Dissolve the protalbic acid in warm water containing ammonium hydroxide, re-precipitate with nitric acid and repeat the solution and precipitation until a pearly, wax-like substance results. This is again dissolved in ammonia, the solution filtered and evaporated and the protalbic acid dried to constant weight at 105° (yield about 160–170 g.). Dry protalbic acid 19 g. in 15% solution is mixed thoroughly with silver oxide freshly precipitated from 100 g. of silver nitrate by means of sodium hydroxide, and washed free from nitric acid by decantation. Add to the mixture 18 g. of sodium hydroxide and 3 to 5 ml. of solution of ammonia (sp. gr. 0.910) and heat on a water-bath at 80° until a portion of the liquid diluted with water is faintly opalescent by reflected light, but clear by transmitted light. The solution is dialysed for two days and then dried in thin layers at 60° to 80°. Yield about 80 g.

For Silver Proteinate. The mother liquor and washings obtained as above are dialysed free from nitrates. The liquid is neutralised to phenolphthalein with sodium hydroxide, and the proportion of lysalbic acid determined by drying a portion of the solution at 105° and ashing the dry powder; the difference between the weights of ash and dry powder gives the lysalbic acid. Lysalbic acid 1 kg., is diluted with water to form an 8% solution to which is added a solution of 155 g. silver nitrate in 500 g. of water. The mixture is heated on a water-bath at 80° until clear, and is then diluted and dialysed for five days. The liquid is evaporated under reduced pressure to the consistence of a thick syrup, solution of hydrogen peroxide is added slowly with constant stirring until the desired light brown colour is obtained, and the product is dried below 60°, preferably under reduced pressure.—R. A. Feldhoff, *Apoth. Ztg.*, 1933, 83, 1205.

ARNICÆ FLOS

Arnicae Flos (B.P.C.). The dried flowerheads of *Arnica montana* Linn. Contains not more than 2% of foreign organic matter, and the percentage of receptacles with their attached involucre is not less than 25 and not more than 33. Yields not less than 15% of alcohol-soluble extractive (45% alcohol) by macerating 5 g. of the air-dried drug, in coarse powder, with 100 ml. of the alcohol for 24 hours, with frequent shaking, filtering and evaporating 25 ml. to dryness and drying at 100°. *Arnica*, *N.F. VII*, should contain not more than 3% of foreign organic matter, and yield not more than 2% of acid-insoluble ash.

Arnicae Rhizoma (B.P.C.). The dried rhizome and rootlets of *Arnica montana* Linn. Should not contain more than 2% of foreign organic matter. Ash, not more than 12%. Yields not less than 14% of alcohol-soluble extractive (0% alcohol).

ARSENUM

Arseni Triiodidum (B.P.). $\text{AsI}_3=455.7$. As determined by titration with N/10 iodine in presence of sodium bicarbonate, it contains not less than 99.0% of the pure substance. At 100° it should not lose iodine, and on volatilisation not more than 0.5% of residue remains. **Arseni Triiodidum, N.F. VII**, after drying to constant weight over sulphuric acid, is of the same strength. **Triiodure d'Arseñic, Fr. Cx.** 1937, assayed for iodine and for arsenic content, contains not less than 99% of AsI_3 .

Liquor Arseni et Hydrargyri Iodidi (B.P.) contains 0.95 to 1.05% w/v of red mercuric iodide and total arsenic equivalent to 0.95 to 1.05% w/v of arsenic triiodide. Assayed for arsenic by addition of sodium bicarbonate, exactly oxidising the arsenic with N/10 iodine, followed by addition of hydrochloric acid and, after 10 minutes, titration with N/10 sodium thiosulphate. 1 ml. N/10 sodium thiosulphate = 0.02279 g. of total arsenic, calculated as AsI_3 ; mercury is determined by precipitation as sulphide in ammoniacal solution.

Arseni Trioxidum (B.P.). $\text{As}_2\text{O}_3=197.9$. Contains not less than 99.8% of As_2O_3 . Residue on volatilisation, not more than 0.1%. Assayed by dissolving in boiling water and N/1 sodium hydroxide, adding an equal quantity of N/1 hydrochloric acid and then sodium bicarbonate, and titrating with N/10 iodine. Arsenious sulphide should be absent as shown by the substance forming a clear colourless solution in 20 parts of dilute solution of ammonia, which does not become yellow when diluted with an equal volume of water, and acidified with hydrochloric acid. **Arseni Trioxidum, U.S.P. XII**, should contain not less than 99.5% of the pure substance after drying to constant weight at 100° .

Tabellæ Arseni Trioxidi (N.F. VII). Contain 92.5 to 107.5% of the labelled amount of arsenic trioxide, including all tolerances. Assayed by boiling with water, cooling and then adding hydrochloric acid, allowing to stand 2 hours and finally titrating with M/50 potassium iodate, using chloroform as indicator.

Liquor Arsenicalis (B.P.) contains 0.95 to 1.05% w/v of As_2O_3 , as shown by titration with N/10 iodine. **Liquor Acidi Arsenosi, U.S.P. XII**, assayed similarly, contains 0.975 to 1.025% w/v of As_2O_3 .

During the preparation of **Liquor Arsenicalis** nearly half the arsenious oxide combines with the alkali to form meta-arsenite, KAsO_2 , the remainder dissolving uncombined in the meta-arsenite solution. On neutralising with acid the compound is decomposed, the final product consisting simply of a solution of arsenious oxide and potassium chloride.—C. Morton, *Quart. J. Pharm.*, 1933, 4.

Liquor Potassii Arsenitis (U.S.P. XII) contains 0.95 to 1.05% w/v of As_2O_3 .

Ferri Arsenas (B.P.C.). Assayed by titration in diluted sulphuric acid solution with potassium permanganate, it contains not less than 10% of ferrous iron calculated as $\text{Fe}_3(\text{AsO}_4)_2$. **Arséniate de Fer, Fr. Cx.** 1937, contains 29 to 31% of arsenic, by precipitation as magnesium ammonium arsenate, and 32 to 34% of iron.

An official method for the determination of arsenic in iron-arsenic tablets is described in *Methods of Analysis (A.O.A.C., 1940, 615)*.

Sodii Arsenas Anhydrosus (B.P.C.). $\text{Na}_2\text{HAsO}_4 = 185.9$. Loses not more than 2% at 150° and then contains not less than 99.5% of the pure compound. Assayed by interaction with potassium iodide in a 50% hydrochloric acid solution, and titration of the liberated iodine with standard sodium thiosulphate. The crystallised salt of the International Protocol contains 36.85% of arsenic acid. Sodii Arsenas Exsiccatus, *N.F. VII*, is required to contain not less than 98% of Na_2HAsO_4 , after drying to constant weight at 150° ; loss at 150° , not more than 3%; in assaying, the interaction with potassium iodide is allowed to proceed during 15 minutes and at a temperature of 80° , with a concentration of hydrochloric acid of only approximately 30%. Arséniate de Sodium Officinal, *Fr. Cx. 1937*, is the heptahydrate; assayed by precipitation with magnesia mixture, contains not less than 58.4% of the anhydrous substance.

Arsenical Sprays. Lead arsenate is used much for fruit-tree spraying, as is also calcium arsenite. Paris Green, *syn.* Schweinfurth Green, Emerald Green, Mitis Green or French Green, i.e., copper aceto-arsenite, is also used. The sale of arsenical preparations should be limited to registered pharmacists and to persons who obtain a licence for the possession of same.—Sir W. H. Willcox, *Brit. med. J.*, ii/1922, 371.

Lead Arsenate. The specification of the Association of British Insecticide Manufacturers (Bulletin No. 82, Ministry of Agriculture and Fisheries) for lead arsenate (di-plumbic arsenate, PbHAsO_4) requires lead arsenate powder to be in the form of fine powder free from lumps and grit, and to contain not less than 31% of arsenic calculated as arsenic pentoxide, As_2O_5 , not less than 63% of lead oxide (PbO) and not more than 0.5% of water-soluble arsenic expressed as As_2O_5 . A limit test for acidity is also prescribed. Lead arsenate paste consists essentially of a mixture of di-plumbic arsenate and water; it should contain not less than 14% of arsenic (As_2O_5) and not less than 28.4% of lead oxide (PbO). It should comply also with tests for acidity and a limit of 0.5% of water-soluble arsenic.

Arsine in Industry.

Arseniuretted hydrogen is formed chiefly in industrial processes when metals or other reducing bodies containing traces of arsenic are dissolved in acids. It is a colourless gas with a faint odour resembling garlic and this may pass undetected. It is a very poisonous gas with a delayed action, whilst it is also believed to be cumulative in effect. Symptoms develop a few hours after exposure and are even then usually indefinite with a feeling of giddiness, headache, nausea, and possibly severe vomiting. The mortality of diagnosed cases of arsine poisoning is about 30%. Arsine is absorbed by the hæmoglobin of the red blood corpuscles, causing hæmolysis, with the result that the arsenic is oxidised, whilst hæmoglobin and albumen, and occasionally blood, appear in the urine. Later symptoms are a jaundiced skin the rapidity and depth of appearance of which depend on the seriousness of the condition. Fatal results occur from exposure to 2.5 parts in 10,000 for half an hour, whilst exposure to 1 part in 20,000 for 1 hour is dangerous and to 1 part in 100,000 for 12 hours is fatal.

Treatment. On account of the delayed action of this gas there is no question of first aid. Treatment consists in blood transfusion to counteract the severe anæmia and the administration of copious draughts of liquid and alkalis, such as sodium citrate (180 to 240 gr. in 24 hours), to increase the flow of urine and assist in the elimination of arsenic. **Detection and Determination.** Arsine is best detected by mercuric chloride or bromide papers, using lead acetate as a filter for hydrogen sulphide if necessary, when the well-known yellow to orange colour of mercuric arsenide is obtained. (*Toxic Gases in Industry*, No. 9, D.S.I.R.)

Arsine Derivatives.

Many cases of poisoning have been reported due to wallpapers containing arsenic and one has been recorded, where the wall was composed of coke breeze and cement. In every instance the walls were damp and it has been shown that the liberation of arsenic is due to the action of mould growths and the formation of substituted arsines, of which the dimethyl and trimethyl and the diethyl arsines are the most important. These gases possess a strong odour of garlic and it has been suggested that the production of trimethylarsine by the growth of *Penicillium brevicaulis* on media, to which has been added a small quantity of the material to be examined, is as sensitive as the Gutzeit test.

At the present time arsenical derivatives are considered primarily as war gases and some of them were actually employed towards the end of the 1914-18 war. In the main, however, it has been decided that only organic derivatives of arsine are likely to be useful and it is doubtful whether more than seven of these are likely to be employed intentionally. The aliphatic compounds are: methylchloroarsine (*Methyl Dick*), ethylchloroarsine (*Dick*), and Chlorovinylarsines (*Lewisite*). The aromatic arsines are: Phenylchloroarsine, diphenylchloroarsine, and diphenylcyanoarsine. One heterocyclic arsine may be employed: diphenylaminechloroarsine. Ethylchloroarsine ($C_2H_5AsCl_2$) was used to a slight extent by the Germans in the last war in an attempt to combine the vesicant properties of mustard gas with the lung irritant properties of phosgene. It is an extremely irritant gas. The methyl compound was not used in the last war, but is similar in behaviour to the ethyl compound, though less than half as toxic.

Lewisite, (2-chlorovinyl dichloroarsine, $ClCH=CH\cdot AsCl_2$) was being manufactured in America in the last war, but has not been actually employed in war. In the pure state it is a colourless, oily liquid. M.p., -13° ; b.p., 190° (decomp.). In the impure state it is a heavy brown liquid with an odour strongly reminiscent of geraniums. It is soluble in ordinary organic solvents and is hydrolysed by water and alkalis. It can be detected fairly readily by hydrolysing with 10% caustic soda, when acetylene is given off and sodium arsenite is formed. If the acetylene is allowed to pass through or to diffuse over a freshly prepared solution containing 1.5% copper sulphate, 3% ammonium chloride, 20%

ammonium hydroxide solution 10%, and hydroxylamine sulphate 5%, a brick-red precipitate of copper acetylide is formed. The arsenic in the remaining solution can be reduced by the modified Gutzeit reaction and detected as mercuric arsenide. Lewisite is a sensory irritant and capable of producing burns similar to those of mustard gas, but irritation is produced almost immediately by liquid on the skin and blisters develop much more rapidly than in the case of mustard gas. The burns heal more rapidly than do those caused by mustard gas, though, if extensive, they may be complicated by the danger of arsenical poisoning. In the production of Lewisite the di- and tri-vinyl arsines are almost always produced. Monochlorovinyl dichloroarsine and dichlorovinyl monochloroarsine possess very similar physiological action, but the trichlorovinyl arsine has practically no aggressive action.

Determination of organic arsines in foodstuffs, etc. The only organic arsine which is readily decomposed is Lewisite and as this may be impure or mixed with another arsenical it is necessary to break down the contaminated material by organic combustion, which converts the arsenic to the arsenical condition, which must be reduced by sulphur dioxide or other process before the arsenic can be determined by the modified Gutzeit process.

TESTS FOR ARSENIC

The modified Gutzeit Test is used in the B.P. '32 and B.P.C. '34 with precise directions, and a list of limits of arsenic in the substances to be tested is given in parts per million.

Modification of Gutzeit Test. A new apparatus in which the tube containing the mercuric chloride paper is surrounded by a cooling system. Increased evolution of hydrogen is obtained by adding a ferrous salt and stannous chloride to the zinc and acid.—H. E. Crossley, *J. Soc. chem. Ind., Lond.*, 1936, 272T.

Marsh's Test consists in generating hydrogen electrolytically or by means of acid and zinc, and adding the substance to be tested. If arsenic be present arseniuretted hydrogen is evolved, and deposits metallic arsenic in the cooler parts of the delivery tube, which is heated at a suitable point.

The sensitiveness of zinc is invariably increased by the use of cadmium sulphate and the use of this salt must be regarded as an essential and inseparable feature of the Marsh-Berzelius process.

Both Marsh's Test and the B.P. method are unreliable. Accurate results may be obtained by the method of Martin and Pien (*Bull. Soc. chim. Fr.*, 1930, 646).—Ch. Bertin, *Ann. Falsif.*, 1938, 215.

Estimation of arsenic especially with regard to determination in the tissues. Zinc is unsatisfactory. The arsenic, e.g., in a Marsh, does not appear until the experiment has proceeded for a period of time. The suggestion that there may be deposits or nuclei of arsenic in impure zinc is certainly good. The electrolytic apparatus of Thorpe is preferable to Marsh.—G. Roche Lynch, *Lancet*, ii/1922, 629.

Keratin tissues take up a proportionately greater amount of arsenic than other tissues of the body.—Prof. Sydney Smith, Sect. of Forensic Medicine, B.M.A. Cent. Meeting, 1932, *Brit. med. J.*, ii/1932, 320.

Reinsch's Test consists in heating the substance with concentrated hydrochloric acid and copper foil; the arsenic is deposited as arsenide on the foil from which it may be sublimed after washing and drying.

The Reinsch test forms a useful basis for a rapid sorting test where a large number of samples of foodstuffs are to be examined for arsenic by the Gutzeit or electrolytic technique. The method recommended is to dilute, if necessary, a suitable aliquot portion of the reduced and diluted acid digest from the nitric acid and sulphuric acid oxidation of a known weight of foodstuff to 7 ml.

with water, and add 3 ml. of conc. hydrochloric acid. The liquid is boiled for not less than 2 minutes with a flat strip of copper foil, which has been previously cleansed by boiling with hydrochloric acid (1 : 1) and rinsed with water. The foil is then washed with water, marked for identification and placed in a shallow white dish containing N/4 hydrochloric acid. It is compared with similarly prepared standards.—J. G. A. Griffiths, *Analyst*, 1941, 491.

Biological Test. The addition of a substance containing a trace of arsenic to a growing culture of *Penicillium brevicaulis* causes in a few minutes an evolution of arsine which can be detected by the smell. 0.0000001 g. of As can be detected.—B. Gosio, *Boll. Ist. sieroter. Milano*, 1932, 61, 597.

The gas evolved is trimethylarsine.—F. Challenger *et al.*, *J. chem. Soc.*, 1933, 95.

The second report of the Commission Internationale des Spécialités describes the following three agreed methods for the determination of arsenic. The method of Schulek and v. Vilecz (*Z. anal. Chem.*, 1929, 76, 81) is applicable to most preparations. The more laborious Schneider distillation process, while of general application, is recommended especially for the determination of arsenic in preparations containing iron. The method of *P. Ned. Supp. I.* is recommended for use with derivatives of arspenamine.—*Bull. Féd. int. Pharm.*, 1938, 36.

Determination by a Continuous Electrolytic Method. A complete and detailed description is given of the apparatus and method of procedure which has been of considerable value in a food factory where a large number of arsenic tests are carried out.—H. C. Lockwood, *Analyst*, 1939, 657.

ORGANIC ARSENIC COMPOUNDS

Acetarsol (*B.P. Add. I*). $C_8H_{10}O_5NaAs = 275.0$. Contains 27.0 to 27.4% of As. Loss at 100° for 4 hours, not more than 0.5%; ash, not more than 0.2%. Assayed for arsenic by the method given for Tryparsamidum. Limit tests for inorganic arsenates and chlorides are included. The free amino-acid present, as indicated by the colour produced on diazotising, should not be greater than that produced by one-thousandth the quantity treated with the same reagents after boiling with hydrochloric acid and water and cooling. Acide *m*-acétylamino-*p*-hydroxyphénylarsinique, *Fr. Cx.* 1937, assayed for arsenic content by digestion with sodium hydroxide and sodium persulphate solution, addition of potassium iodide to the acidified mixture and titration with N/10 sodium thiosulphate, contains 27 to 27.5%. *m*-Acétylamino-*p*-hydroxyphénylarsinate de Sodium, *Fr. Cx.* 1937, assayed as for Chlorhydrate de Di-amino-di-hydroxy-arséno-benzène, contains 19 to 19.5% of arsenic.

Arsphenamina (*B.P.C.*). Complies with biological tests, carried out in an approved institution or laboratory, for maximum toxicity and therapeutic potency (*vide infra*) and contains, when determined by an approved method, from 30 to 34% of As. It is controlled by regulations made under the Therapeutic Substances Act, 1925, the standard preparation for Great Britain and Northern Ireland being kept in the National Institute for Medical Research, London. The percentage of As may be determined by the *B.P.C.* method for Sodii Aminarsonas. When kept in sealed phials at a temperature of 56° for twenty-four hours, it retains its colour, physical properties and solubility. It should comply with tests for solubility in water, a sodium hydroxide solution and a sodium chloride solution. Arsphenamina, *U.S.P. XII*,

complies with the requirements of the National Institute of Health, United States Public Health Service, and contains 30 to 32% of arsenic. Assayed by digesting for 10 minutes with five times its weight of powdered potassium permanganate and 25 volumes of diluted sulphuric acid and mixing well; a further 50 volumes of concentrated sulphuric acid is then added in small portions, the brown precipitate dissolved with just sufficient hydrogen peroxide, diluted and boiled to expel the excess; sufficient potassium permanganate is added to produce a pink coloration, this being decolorised with a drop of N/10 oxalic acid; the cooled solution is then set aside in the dark for 1 hour with potassium iodide when the liberated iodine is titrated with N/10 thiosulphate; a blank titration is made. All arsphenamine labels must bear an expiration date, which is not more than 5 years from the date of manufacture. 0.1 g. in 10 ml. of water requires 3.9 to 4.3 ml. of N/10 sodium hydroxide for neutralisation to phenolphthalein. The ampouled product shows no change in colour, consistency or solubility when kept at 70° for 48 hours. Dichlorhydrate de Diamino-di-hydroxy-arséno-benzène, *Fr. Cx.* 1937, contains not less than 30% of As and not more than 3% of S. Tests for toxicity on rabbits and mice are included.

Tests for Arsphenamine: Recognition in Medico-Legal Cases. The behaviour of arsphenamine with the usual reagents for arsenic has been investigated to find a method of distinguishing between it and inorganic arsenic in medico-legal cases.

Muscle from a patient who had died three weeks after injections of arsphenamine still gave reactions for arsenic. The drug gives the Reinsch, Marsh, Gutzeit (after oxidising and reducing) and biological tests for arsenic. The following serve to distinguish arsphenamine from inorganic forms of arsenic. With Bettendorf's reagent it gives an amorphous, yellow ppt. which dissolves on warming and reappears on cooling. H_2S gives no ppt. even after a solution of the drug has been boiled with HCl. The organic part of the arsphenamine molecule gives certain reactions, which may afford confirmatory evidence of the presence of the drug, thus:—The corresponding diazo-derivative gives a characteristic red to violet precipitate with α -naphthylamine, which may be isolated and examined for arsenic by the Reinsch or Gutzeit test. Sodium aminarsonate behaves similarly, giving a red azo-dye, but the diazotised arsphenamine gives no colour with β -naphthylamine, whilst sodium aminarsonate gives a vermilion-red azo colouring matter with the β -amine.

Minced horseflesh sprayed with arsphenamine solution and kept for 14 days was extracted with alcohol, slightly acidified with HCl. The residue so obtained gave positive results with the Reinsch, Gutzeit, and α -naphthylamine tests, but negative results with Bettendorf's reagent, and with hydrogen sulphide. So far it has proved impossible to obtain good results by applying to arsphenamine ordinary toxicological methods for the estimation of arsenic, the latter being obtained only to the extent of from 29 to 29.5% out of the 34% present.—*J. chem. Soc. Abstr.*, ii/1911, 448. See also Sir W. H. Willcox, *Brit. med. J.*, i/1916, 474.

Estimation of the arsenic excreted in the urine has been conducted, the general conclusions being: (a) the elimination begins rapidly; (b) the duration of the passing of arsenic in the urine is longer than was thought; (c) after subcutaneous injection the elimination is concluded more rapidly than by the intramuscular method; (d) simultaneous use of mercury caused delay in eliminating the arsenic; (e) potassium iodide given at the same time shortens the duration of the arsenic elimination.

Biological Tests.

Arsphenamine must be tested for toxicity and therapeutic activity. The Regulations under the Therapeutic Substances Act, 1925, do not give directions for these tests, but the directions are supplied on application to the Ministry

of Health. The directions describe the tests carried out in the National Institute for Medical Research, London.

(a) **Toxicity Test.** This is carried out on mice of uniform weight. 0.1 g. of arsphenamine is weighed and dissolved in about 6 ml. of water. The water is freshly distilled, being condensed and collected in glass vessels. 0.45 ml. of 2N sodium hydroxide is added, the liquid shaken and diluted to 10 ml. With this 1% solution five mice are injected with a dose corresponding to 0.1 mg. per g., and five mice with a dose of 0.125 mg. per g. The injections are made into the tail vein. The mice are observed during 3 days. The sample is regarded as not unduly toxic provided that at least three mice out of five have survived each dose.

(b) **Test for Therapeutic Potency.** The curative action of each sample is tested on mice infected with a species of trypanosome. *Trypanosoma equiperdum* is commonly used because it is non-pathogenic to man. This strain of trypanosome is carried for long periods in guinea-pigs and is transferred to rats by injecting some of the guinea-pigs' blood into the peritoneal cavity. When a test is to be carried out some thirty mice are infected from the blood of an infected rat. The trypanosomes in the rat's blood are counted and each mouse receives 0.5 ml. of a suspension containing a certain number of organisms. After 2 days the trypanosomes in the blood of each mouse are counted. The haemocytometer apparatus for counting red blood cells is used, the trypanosomes being stained with methyl violet. Those mice are used for the test which contain in their blood not less than 100,000 and not more than 500,000 trypanosomes per cu. mm. of blood. At least ten mice are then injected by way of the tail vein with the sample being tested, a dose of 0.01 mg. per g. being given; at least ten mice are also injected with the standard sample of arsphenamine in the same dose. The blood of the mice is then examined daily for the next four days to discover the rate of disappearance of trypanosomes. A sample is only accepted as satisfactory if its curative action is at least as quick as that of the standard and is seen in as large a proportion of mice.

Arsphenamina Argentica (B.P.C.). Controlled by regulations made under the Therapeutic Substances Act and complies with biological tests carried out in an institution or laboratory approved by the licensing authority for maximum toxicity and therapeutic potency (*vide infra*). By an approved method, such as that given under Sodii Aminarsonas, it contains not less than 18% and not more than 21% of As; it contains not less than 12% and not more than 13% of Ag, estimated by an approved method. The 5% aqueous solution should be almost black, and alkaline to litmus, and on the addition of sodium hydroxide or sodium carbonate no precipitate is produced, but with sodium bicarbonate a precipitate appears. It may be assayed for Ag by adding ammonium persulphate to an aqueous solution and boiling until colourless; diluting, adding nitric acid and titrating with N/50 ammonium thiocyanate to ferric ammonium sulphate indicator.

For the toxicity test, doses corresponding to 0.125 mg. and to 0.15 mg. per g. body weight are injected in 1% solution into the tail vein of each of five mice. Three mice must survive each dose. For the therapeutic test a 0.05% solution is injected into infected mice. Five receive 0.009 mg. per g. body weight and five receive 0.008 mg. The tests are performed as for arsphenamine.

Neoarsphenamina (B.P.). Controlled by regulations made under the Therapeutic Substances Act and complies with biological tests, carried out in an approved institution or laboratory, for maximum toxicity and therapeutic potency (*vide infra*). The standard preparation of neoarsphenamine is kept in the National

Institute for Medical Research, London. It complies with a test for solubility, and absence of arsphenamine. Kept in sealed phials for 24 hours at 56° it retains its colour, physical properties and solubility. Neoarsphenamina, *U.S.P. XII*, complies with the requirements of the United States Public Health Service and should contain not less than 19% of As, determined by the *U.S.P. XII* process for Arsphenamina. All neoarsphenamine labels must bear a date beyond which the substance must not be used, this date being not more than 3 years from the time of manufacture. Loss on drying for 24 hours in a vacuum desiccator over fresh phosphorous pentoxide, not more than 1.5%. The *Fr. Cx.* 1937 requires the substance to comply with toxicity tests, to contain not less than 19% of arsenic and not more than 12% of sulphur.

Biological Tests.

Neoarsphenamine is tested for toxicity and potency by tests similar to those given for arsphenamine.

(a) **Toxicity test.** An important study of the toxicity of neoarsphenamine has been published by Durham, Gaddum and Marchal (*Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 128, 1929). They determined the curve relating the percentage of mice killed to the dose injected, obtaining a curve for mice of 14 g. and also for mice of 19 g. The dose of the international standard neoarsphenamine which kills 50% of mice of 14 g. weight is 8.1 mg. By international agreement samples are allowed to pass the test if they do not exceed the toxicity of the standard by more than 20%, hence it would be expected that the dose of a sample to be

injected would be $\frac{5}{6} \times 8.1$ or 6.75 mg. Actually the requirement in Gt. Britain is more lenient than this, for the dose to be injected is only 6 mg., from which it follows that samples exceeding the toxicity of the standard by 35% are accepted. Apparently no country imposes a more severe standard than this despite the international agreement to do so. The test requires a dose of 6 mg. in 2% solution to kill not more than 15 out of 30 mice of 13 to 15 g. weight. Experience has shown that some samples which pass this test are more toxic when injected in stronger solution, and therefore a subsidiary test in which a 5% solution is used is also performed. A dose equivalent to 0.225 mg. per g. is injected into five rats, of which not more than one may die if the sample is to be approved.

(b) **Therapeutic test.** The test is similar to that for arsphenamine. Five infected mice receive 0.03 mg. per g. as a 0.2% solution, and 5 mice receive 0.025 mg. per g. Similar doses of the standard are also given to the same number of other mice.

Variations in Toxicity and Trypanocidal and Spirochæticidal Properties. 18 samples, prepared by 7 different laboratories, of neoarsphenamine purchased on the open market were found to vary considerably. The maximum tolerated dose for white rats varied from 0.200 g. to 0.300 g. per kilo of weight; the average minimal trypanocidal dose varied from 0.004 to 0.008 g. per kilo weight, 28% being incompletely trypanocidal; and with the average minimal spirochæticidal dose for rabbits with acute syphilitic orchitis of 0.020 g. per kilo, 14 samples were completely spirochæticidal in this dose while 6 were not. The establishment of standards urged.—J. F. Schomberg and J. A. Kolmer, *J. Amer. med. Ass.*, i/1933, 183.

Neoarsphenamine having a low toxicity need not have a low trypanocidal activity. Most of a number of brands assayed, which showed a low toxicity had a high trypanocidal potency, and the most toxic were usually, although not invariably, the least active.—C. A. Morrell and M. G. Allmark, *J. Amer. pharm. Ass., Sci. Edn.*, 1941, 33.

Sulpharsphenamina (B.P. Add. I). Controlled by the regulations made under the Therapeutic Substances Act, 1925. The standard preparation is kept in the National Institute for

Medical Research, London. It complies with biological tests, carried out in an institution or laboratory approved by the licensing authority, for maximum toxicity and therapeutic potency (*vide infra*). It should comply with a test for absence of arsphenamine, and should retain its colour, physical properties and solubility when kept in sealed phials at 56° for 24 hours. The test for distinction from neoarsphenamine requires no precipitate to be produced on the addition of dilute hydrochloric acid to an equal volume of solution of the substance (1 in 3). The sterile solution should be used immediately after preparation. Sulpharsphenamina, *U.S.P. XII*, contains not less than 19% of As, when assayed by the *U.S.P. XII* method for Arsphenamina. 0.6 g. dissolves completely in 3 ml. of distilled water within 5 minutes. The label on the container complies with the regulations given under arsphenamine and there is a similar test for the ampouled product. The substance of the *Fr. Cx.* 1937 should contain 18 to 20% of arsenic, not more than 15% of sulphur and complies with toxicity tests and tests for trypanocidal power.

The biological tests for sulpharsphenamine are similar to those for neoarsphenamine except that the doses are given by subcutaneous injection. For the toxicity test on mice, the volume injected into each mouse of 14 g. is 0.35 ml. of a 2% solution; for the subsidiary test on rats each rat receives 0.35 mg. per g. in 10% solution. For the therapeutic test, five mice receive 0.05 mg. per g. and five mice receive 0.04 mg. per g., both given as a 0.2% solution.

Sodii Aminarsonas (B.P.C.). $C_6H_7O_3NaAsNa = 239.0$. Contains from 24 to 25.6% of As, when assayed by the method of the *B.P.C.* '34: 0.2 g., accurately weighed, is heated for 1 hour with 5.5 ml. of sulphuric acid and 1 ml. of fuming nitric acid; to the slightly cooled product 15 drops of fuming nitric acid are added and heat applied for a further 5 minutes; 1 g. of ammonium sulphate is cautiously added and after cessation of evolution of nitrogen, cooling, and diluting to about 70 ml., 1 g. of potassium iodide is added and the liquid concentrated to about 40 ml.; after decolorisation with just sufficient N/100 sodium thiosulphate and dilution to about 110 ml., the addition of 50 ml. of 4N sodium carbonate and a slight excess of sodium bicarbonate, for neutralisation, the mixture is titrated with N/10 iodine, using starch mucilage as indicator. Anilarsinate de Sodium, *Fr. Cx.* 1937, the substance with 4 molecules of water, contains not less than 24% of arsenic.

Sodii Cacodylas (B.P.C.). $C_2H_6O_2AsNa_3 \cdot 3H_2O = 214.0$. By the *B.P.C.* method for Sodii Aminarsonas it contains from 72 to 75% of $C_2H_6O_2AsNa$. It complies with limits for acidity or alkalinity to phenolphthalein, disodium methyl arsonate, arsenate and phosphate. The *U.S.P. XII* allows the same limits of purity; assayed by digestion with sulphuric acid and potassium sulphate, cooling and neutralising the solution, rendering faintly acid and titrating with N/10 iodine. Cacodylate de Sodium, *Fr. Cx.* 1937, assayed similarly, contains 73 to 76% of the anhydrous salt; it is also assayed for arsenic by the method given under Arsphenamina. Méthylarsinate de Sodium, *Fr. Cx.* 1937,

contains not less than 25.5% of arsenic. Natrium kakodylicum, *P.G. VI*, $(\text{CH}_3)_2\text{AsO}_2\text{Na} + 3\text{H}_2\text{O}$, contains 32.8 to 35% of arsenic; assayed by addition of 5 ml. of water, 10 ml. of sulphuric acid and 2.5 g. of potassium permanganate to 0.2 g. of substance, strongly shaking and allowing to stand for 20 hours; then after warming on a water-bath at 100° and then on a wire gauze for 12 to 20 minutes, cooling, rinsing the flask with 50 ml. of water and decolorising with crystals of oxalic acid, finally adding 2 g. of potassium iodide and titrating with N/10 sodium thiosulphate. 1 ml. of N/10 sodium thiosulphate = 0.003748 g. of arsenic. Natrium cacodylicum, *P. Helv. V*, by digestion with sulphuric acid and potassium sulphate, diluting and titrating, after addition of hydrochloric acid and methyl orange, with N/10 bromide-bromate solution until the red colour disappears, contains 72 to 75% of the anhydrous substance, equivalent to 0.003748 g. of arsenic. 1 ml. of N/10 KBrO_3 = 0.00800 g. of $\text{C}_2\text{H}_5\text{O}_2\text{AsNa}$ = 0.003748 g. of As.

Tryparsamidum (*B.P. Add. I*). $\text{C}_8\text{H}_{10}\text{O}_4\text{N}_2\text{AsNa}_2 \cdot \frac{1}{2}\text{H}_2\text{O}$ = 305.0. Loses at 110° from 2.5 to 3.5% of its weight and then contains 25.1 to 25.5% of As in organic combination, and 9.25 to 9.5% of N. Assayed for nitrogen by the Kjeldahl process and for As by digestion with sulphuric and fuming nitric acids followed by addition of ammonium sulphate; to the cooled and diluted colourless liquid potassium iodide is added, the solution concentrated and decolorised with N/10 sodium thiosulphate; after rendering alkaline and then acid, sodium bicarbonate is added and the As titrated with standard iodine. Contains not more than 0.25% of arsanilic acid, as shown by comparing the colour produced on diazotising a quantity with addition of arsanilic acid, with that from one half the quantity of substance with twice the amount of added arsanilic acid. Tests for absence of arspenamine compounds, and of arsenate and phosphate are included. The 30% solution should remain clear for 6 hours. The *U.S.P. XII* substance, assayed similarly, is required to comply with the same standard for As. All tryparsamide labels must bear an expiration date, which is not more than 5 years after the date of manufacture. By treatment of 0.5 g. in 45 ml. water with hydrochloric acid and potassium iodide on the water-bath for 20 minutes and, after cooling and addition of excess sodium bicarbonate, titration with N/10 sodium thiosulphate, the *Fr. Cx.* 1937 substance contains 24.4 to 24.9% As.

Test for Arsanilic Acid. Estimation of small amounts of arsanilic acid in tryparsamide can be carried out by the following colorimetric method. Weigh accurately 0.5 g. and 1.0 g. of tryparsamide into two test-tubes and dissolve in 6 ml. of distilled water. Cool each tube below 5° in an ice-bath and add 2.5 ml. of 4% sodium nitrite solution followed by 5 ml. of dilute hydrochloric acid. The solutions are mixed after each addition of reagent. Pour the contents of each tube into 10 ml. of a previously cooled solution of β -naphthol. Mix the diazotised solution and alkaline β -naphthol by pouring from one tube to the other and place in the 1 cm. cell of a Lovibond Tintometer. Match the colour and record the red units. The quantity of arsanilic acid present is determined by reference to the graph included in the paper. The weights of sample taken give a colour of an order to be easily matched, provided

that the sample just satisfies the *B.P.* limit test for arsanilic acid of which the method described is a modification. The critical factor in the estimation is the amount of sodium nitrite and most accurate results are obtained when an amount of tryparsamide containing arsanilic acid sufficient to give a colour between 4.0 and 8.0 red units is used. The Pharmacopœial limit test is satisfactory for tryparsamide containing mere traces of arsanilic acid, but where larger amounts are present it is not possible to determine whether the sample satisfies the test or not.—C. A. MacDonald and J. G. Reynold, *Quart. J. Pharm.*, 1939, 534.

Trypanocidal action and chemical constitution of arsenic compounds.—H. King, W. O. Murch and I. E. Balaban, *J. chem. Soc.*, 1925, 2632, 2701.

ASAFŒTIDA

Asafœtida (*B.P.*). Leaves not more than 50% of matter insoluble on boiling with alcohol (90%) and drying at 100°. Ash, not more than 15%. **Asafœtida**, *N.F. VII*, by continuous extraction with alcohol (94.9%), drying the residue at 100° for 30 minutes and calculating to the dried drug (the moisture having been determined by toluene distillation) yields not less than 50% of extractive; acid-insoluble ash, not more than 15%.

Asafetida Oil has been examined; the main fraction, b.p. 82° to 84° at 10 mm., is optically active, has a composition corresponding to $C_7H_{14}S_2$ and structural formula $CH_3CH_2 \begin{matrix} \searrow \\ CH \cdot S \cdot S \cdot CH : CH \cdot CH_3 \end{matrix}$.—Mannich and Fren-

senius, *Arch. Pharm., Berl.*, 1936, 461, per *Quart. J. Pharm.*, 1936, 710.

Myrrha (*B.P.*). Contains not more than 4% of other organic matter; ash, not more than 9%; matter insoluble in alcohol (90%), not more than 70%. **Myrrha**, *U.S.P. XII*, yields not less than 30% of alcohol-soluble extractive; acid-insoluble ash, not more than 5%.

AZORUBRUM

(and OTHER MEDICINAL DYES)

Azorubrum (*B.P.C. Supp. IV*). $C_{20}H_{12}N_2O_7S_2Na_2 = 502.2$. (Bordeaux B—Colour Index No. 88). Contains 60 to 70% of $C_{20}H_{12}O_7N_2S_2Na_2$. Assayed by the *B.P.* method described for Methylthioninæ Chloridum, dissolving in hot water and continuing the titration with N/10 titanous chloride until the solution is nearly colourless. 1 ml. N/10 titanous chloride = 0.001255 g. of $C_{20}H_{12}O_7N_2S_2Na_2$. Limit of zinc; 0.1 g. ignited with sulphuric acid and the residue dissolved in 2 ml. dilute hydrochloric acid and 10 ml. water, dilute solution of ammonia added, boiled and filtered, on acidifying and adding 1 ml. of potassium ferrocyanide solution, produces not more than a faint turbidity. Bordeaux B is practically unaffected by dilute acids or alkalis or by exposure to sunlight, but in use it must not be confused with other dyes known by the same name.

Magenta (*B.P.C.*). (Colour Index No. 677). The sulphated ash is not more than 5% and that from 1 g. gives no precipitate of zinc ferrocyanide. This is basic fuchsine and should be distinguished from acid fuchsine (Colour Index No. 692).

Carminum (B.P.C.). Loses not more than 15% of its weight at 100°, and then yields not more than 10% of ash. A limit of insoluble matter is included. The *N.F. VII* allows 25% loss on drying at 100°; ash, not more than 12%; tests for tin, lead, and soluble barium compounds and for insoluble salts are included.

Methylthioninæ Chloridum (B.P.). $C_{16}H_{18}N_3ClS = 319.7$. Methylene blue is assayed by titration in boiling aqueous solution acidified with hydrochloric acid, the air in the flask being replaced by carbon dioxide, with N/10 titanous chloride until the blue colour is replaced by a reddish-grey; 1 ml. of N/10 titanous chloride is equivalent to 0.01598 g. of $C_{16}H_{18}N_3ClS$, and a purity of not less than 80% is indicated. The solution on reduction is decolorised with potassium iodide, it produces a deep blue flocculent precipitate, with a pale blue supernatant liquid, and, on oxidation with potassium dichromate, gives a reddish-violet colour and a bluish-violet precipitate, the blue colour being restored with sulphurous acid. A limit of zinc is included. Methylthioninæ Chloridum, *U.S.P. XII*, is the trihydrate ($C_{16}H_{18}N_3ClS \cdot 3H_2O$) and loses not more than 18% at 110°; ash, not more than 1.2%; the dried salt contains 98.5% of the pure anhydrous substance. In the revised assay process a cooled solution of the substance is precipitated with potassium perchlorate solution, the residue filtered and washed with saturated methylthionine perchlorate solution, and dried at 110°.

In the absence of B.P. limits for moisture and ash, 20% of the material may be neither water nor methylene blue.—*Pharm. J.*, ii/1932, 128.

An official quantitative method for the determination of methylthionine chloride in tablets and capsules is described in *Methods of Analysis* (A.O.A.C., 1940). Clean, if necessary, a solution of the material containing about 0.1 g. to 0.14 g. of methylene blue with carbon tetrachloride or with dichlorhydrin, shake well with 50 ml. of glacial acetic acid and allow to stand for 25 minutes. Add 30 ml. of N/5 iodine, at first about one-third by fast drops and the remainder quickly, shake and allow to stand for 50 mins., shaking occasionally. Adjust to 200 ml., stand for a further 10 minutes, filter rapidly and then titrate an aliquot portion of the filtrate with N/10 thiosulphate and make a blank determination; 1 ml. of N/5 iodine = 0.01495 g. of methylene blue.

For the use of methylene blue as a renal function test, see p. 663.

Methylrosanilinæ Chloridum (U.S.P. XII). Loses not more than 7.5% at 110°; ash, not more than 1.5%; limit of dextrin, 1%. A limit of lead is prescribed.

Methylviola (B.P.C.). (Colour Index No. 680). Methyl violet yields not more than 5% of sulphated ash, of which that from 1 g. gives no precipitate of zinc ferrocyanide in presence of hydrochloric acid. Limit of dextrin insoluble in boiling alcohol, not more than 1%.

Novaurantia (B.P.C.). $C_{14}H_{10}N_2O_7S_2Na_3 = 452.2$. (Colour Index No. 27). Orange G yields not less than 36% and not more than 50% of sulphated ash. The sulphated ash from 1 g. gives no precipitate of zinc ferrocyanide.

Phenothiazina (N.F. VII). $C_{15}H_9NS = 199.26$. When dried at 100°, loses not more than 0.5% and contains not less than 95% $C_{15}H_9NS$. Phenothiazine melts between 181° and 185° and is assayed colorimetrically after oxidation with bromine, by comparison against a test solution of phenothiazine melting between 184° and 185°.

Rubrum Scarlatinum (B.P.C.). (Colour Index No. 258). Scarlet red yields not more than 10% of sulphated ash. Rubrum Scarlatinum, *N.F. VII*, yields not more than 1% of ash and not more than 1% of water-soluble substances dried at 100°.

Tartrazina (B.P.C.). $C_{16}H_8O_5N_4S_2Na_2 = 534.2$. (Colour Index No. 640). Leaves not more than 80% of sulphated ash, which complies with the limit test for zinc.

Viola Crystallina (B.P.C.). (Colour Index No. 681). Crystal violet yields not more than 5% of sulphated ash, which should comply with the limit test for zinc.

Distinction from Methyl Violet. 2 ml. of a 0.1% aqueous solution of the dye is treated with 8 ml. of alcohol and 5 ml. of sodium hydroxide solution; after standing for 2 minutes the solution should be clear and colourless.—F. Reimers, *Dansk. Tidsskr. Farm.*, 1937, 52.

Viride Malachitum (B.P.C.). (Colour Index No. 657). Malachite green yields not more than 1% of sulphated ash, which should comply with the limit test for zinc.

Viride Nitens (B.P.C.). (Colour Index No. 662). $C_{27}H_{22}N_4SO_4H = 482.3$. Brilliant green yields not more than 5% of sulphated ash which complies with the limit test for zinc.

Indicarminum (B.P.). $C_{16}H_8O_8N_2S_2Na_2 = 466.2$. Indigo carmine is assayed by titration with N/10 potassium permanganate in warm solution acidified with an equal volume of dilute sulphuric acid, the colour changing from green to pale yellow; each ml. of N/10 potassium permanganate is equivalent to 0.0133 g. of $C_{16}H_8O_8N_2S_2Na_2$ of which not less than 90% should be indicated, calculated on the substance dried at 100°. Loss at 100°, not more than 10%, and the residue on ignition and re-ignition with sulphuric acid is then not less than 30% and not more than 40%. The *Fr. Cx.* 1937 assays by treatment with nitric acid and ignition and requires the residue (mostly sodium sulphate) to be 33 to 38%.

For the use of indigo carmine as a renal function test see p. 663.

Indigo or Indigotin (natural) is obtained from the shoots of *Indigofera tinctoria* (Leguminosæ) in India and Java, by maceration with lime and water. The pure substance has the composition $C_{16}H_{10}N_2O_2$.

Indigo Soluble, as mostly understood, is the acid substance, not the sodium salt.

Isatin. $C_8H_5NO_2$. Yellowish-red prismatic crystals obtainable by oxidising indigo with chromic or nitric acid, also by boiling *o*-nitrophenylpropionic acid with caustic soda.

Litmus is a blue pigment obtained from *Rocella tinctoria* (Discomycetes). Employed chiefly as an indicator for acid and alkali as Litmus Paper, also in form of solution in volumetric analysis. Litmus is made in Holland by fermenting lichens in presence of ammoniacal liquids and potash.

LITMUS SOLUTION (see Indicators, p. 606).

In titration, all CO_2 must be removed by boiling before taking end reaction. Not suitable for weak bases. Quinine, morphine and strychnine are neutral to it and the acids in their salts can be titrated as if base were absent.

Carbon dioxide only turns litmus "wine red" when alkaline bicarbonates are present as impurities, otherwise it turns it red just like any other acid.

Lacmoid, also known as Resorcin Blue, is chiefly diazo-resorcin. Solution (0.2% in dilute alcohol), employed as indicator, closely resembles litmus in reactions.

Cudbear, syn. Red Indigo, a purplish-red powder obtained by the ammoniacal fermentation of *Lecanora tartarea* and other lichens, designated in Germany *Persio*, in France *Orseille de terre*. Except for the fact that it is in the condition of a fine powder it is virtually the same as orchil. *Persio, N.F. VII*, yields not more than 12% of total ash; the depth of colour of the alcohol- and water-soluble matter from 0.005 g. approximates that of 1.5 ml. of 5.95% cobaltous chloride solution in hydrochloric acid and water with 3 ml. of N/200 potassium dichromate and 30 ml. of ammonium carbonate solution.

Archil, syn. Orchil. The word Archil, or more properly Orchil, was originally the name of the plant from which the dye, which goes under this name, is obtained. It appears that before the introduction of orchil into this country a similar dye obtained from certain lichens in Scotland was in use under the name "Cork." This is given in Miller's "Plant Names" as the name of the lichens yielding archil.

Turnsole, syn. Tournesol (French). The familiar colouring used on Dutch cheeses. The word has been more particularly applied to a product from *Chrozophora tinctoria* A. Juss (*Croton tinctorius* Linn.)—a native of Southern Europe and the Orient. Rags soaked in the juice of this plant are exported to Holland. They change colour on exposure to ammonia vapour, and this purple colour can be extracted with water for the purpose in question. Turnsole was at one time supposed to form the colouring matter of litmus.

BALSAMUM PERUVIANUM

Balsamum Peruvianum (B.P.). Balsamic esters, determined by extraction with ether in alkaline solution, evaporation and drying at 100° for 30 minutes, not less than 53% and having a saponification value of not less than 235. Balsamum Peruvianum, *U.S.P. XII*, by a similar but aliquot part method, yields 50 to 60% by weight of cinnamein, having a saponification value of 230 to 240; acid number, 56 to 84. Baume de Pérou, *Fr. Cx.* 1937, contains 52 to 56% of cinnamein.

Various qualitative tests for the detection of adulteration are described.—E. M. Smelt, *Quart. J. Pharm.*, 1932, 378.

The greater part of the alcohol peruvial which was considered to be a constituent has been shown to consist of nerolidol, a sesquiterpene alcohol $C_{15}H_{24}O$, also present in oil of neroli.—*Mnfg. Chem.*, 1940, 244.

Balsamum Tolutanum (B.P.). Free balsamic acid content, 19 to 25%, and total balsamic acid content, 35 to 50%, both calculated with reference to the dried alcohol-soluble matter. Assayed by precipitation, for free balsamic acids in an alcoholic potash solution and total balsamic acids in a saponified solution, with magnesium sulphate, filtration and extraction of the acidified filtrate with ether, transferring to sodium bicarbonate solution, re-extraction with ether and drying in a vacuum desiccator over sulphuric acid. Acid value, by a specified process, 97 to 160; ester value, 47 to 95; saponification value, 170 to 224—all calculated on the dry alcohol-soluble matter. Matter insoluble in alcohol, not more than 4%. Loss *in vacuo* over sulphuric acid, not more than 4%. The *U.S.P. XII* substance has an acid number of 112 to 168 and a saponification value of 154 to 220.

Benzoinum (B.P.). Sumatra benzoin only is official. The dry alcohol-soluble matter yields from 19 to 29% of free balsamic acids, and 30 to 60% of total balsamic acids, and has an acid value of 115 to 163, an ester value of 47 to 83, and a saponification value of 169 to 223. Alcohol-insoluble residue, dried at 100°, not more than 20%. Loss *in vacuo* over sulphuric acid, not more than 10%. Ash, not more than 2%. The *U.S.P. XII* requires Sumatra benzoin to yield 75% of alcohol-soluble extractive and not more than 1% of acid-insoluble ash, and Siam benzoin, not more than

1% of foreign organic matter, 90% of extractive, and not more than 0.5% of acid-insoluble ash. Alcohol-soluble extractive determined by continuous extraction for 5 hours, with the addition of sodium hydroxide to the receiving flask, weighing the insoluble residue and subtracting this plus the moisture determined by toluene distillation from the original weight taken; the *U.S.P. XII* deletes the test for rosin included in the *U.S.P. XI*.

The *B.P.* ash limit is too stringent and the acid and ester values will exclude many genuine samples.—E. J. Parry, *Chem. & Drugg.*, ii/1932, 251.

Styrax (*B.P.*). Loses in one hour on a water-bath not more than 5% of its weight and then contains not less than 30% of total balsamic acids, has an acid value of 55 to 90, an ester value of 100 to 133, and a saponification value of 170 to 200. Assayed by the process for total balsamic esters as given under *Balsamum Tolutanum*. *Styrax, U.S.P. XII*, is not the purified product and both the Levant and American varieties are official. After purification it has an acid value of 50 to 85 (Levant storax) or 36 to 85 (American storax), a saponification value of 160 to 200, and yields not less than 25% of cinnamic acid; determined by saponification and evaporation of the neutralised liquid, followed by cleaning of the dissolved residue with ether, acidifying and extracting with ether; after evaporating the ether, the residue is refluxed with boiling water, the hot filtered solution cooled to 25°, the separated cinnamic acid collected on a Gooch, and dried at 80°.

BARBITONUM

Barbitonum (*B.P.*). $C_8H_{12}O_3N_2 = 184.1$. M.p., 189° to 192°. Ash not more than 0.05%. Neutral and basic substances, not more than 0.1%. *Barbitalum, U.S.P. XII*, yields not more than 0.1% of ash; m.p., 188° to 192°.

Tabellæ Barbitali (*U.S.P. XII*). Contain 94 to 106% of the labelled amount of $C_8H_{12}N_2O_3$, including all tolerances. Assayed by dissolving a weighed quantity of powdered tablets in an aqueous solution of sodium hydroxide (1 in 50) saturated with sodium chloride, extracting with ether and rejecting the ether extracts, then acidifying with hydrochloric acid and extracting the barbitone with alcohol-ether-chloroform mixture (2:1:7). The extracts are washed with faintly acid water and the aqueous washings are extracted with ether. After filtration of the combined solvent and ether extracts, the organic solvents are removed, the residue dried at 100° for 2 hours and weighed.

An official quantitative method for the determination of barbitone or phenobarbitone in tablets is described in *Methods of Analysis (A.O.A.C., 1940, 574)*.

Barbitonum Solubile (*B.P.*). $C_8H_{11}O_3N_2Na = 206.1$. Yields, by extraction with ether from acidified solution and drying at 100°, barbitone equivalent to not less than 97% of the pure sodium salt. The *U.S.P. XII* requires *Barbitalum Sodicum* to lose not more than 1% at 100°. Yields 88 to 90% barbitone, calculated on a moisture-free basis. *Barbitalum solubile, P. Helv. V*, by titration in aqueous solution to methyl orange with N/10 hydrochloric acid, contains not less than 97% of $C_8H_{11}O_3N_2Na$.

Tabellæ Barbitali Sodici (U.S.P. XII). Contain 94 to 106% of the labelled amount of $C_5H_4N_2O_3Na$, including all tolerances. Assayed by the process described for barbitone tablets.

Hexobarbitonum (B.P. Add. III). $C_{12}H_{16}O_3N_2 = 236.1$. M.p., 145° to 147° . Neutral and basic substances, not more than 0.1%. Ash, not more than 0.05%.

Hexobarbitonum Solubile (B.P. Add. III). $C_{12}H_{15}O_3N_2Na = 258.1$. Contains, by titration with N/10 sulphuric acid to methyl red, a percentage of sodium equivalent to 8.86 to 9.0% of the $C_{12}H_{15}O_3N_2Na$; yields by extraction with chloroform from the acidified liquid from the sodium titration, and drying at 100° , hexobarbitone equivalent to not less than 96% of the pure substance. Neutral and basic substances, not more than 0.1%.

Pentobarbitalum Sodicum (U.S.P. XII). $C_{11}H_{17}O_3N_2Na = 248.15$. Calculated on the anhydrous substance, it contains 90 to 92% of pentobarbital ($C_{11}H_{18}O_3N_2$). Loses not more than 5% during 6 hours at 90° . Assayed by extraction with ether from acidified solution, finally drying at 90° .

Tabellæ Pentobarbitali Sodici (U.S.P. XII). Contain 90 to 105% of the labelled amount of $C_{11}H_{17}O_3N_2Na$, including all tolerances. In the assay, a weighed quantity of the powder obtained from not less than 20 tablets is dissolved in 2% aqueous sodium hydroxide solution saturated with sodium chloride. After preliminary extraction with ether, the solution is acidified with hydrochloric acid and extracted completely with alcohol-ether-chloroform mixture (2:1:7). The combined extracts are washed with faintly acid water, filtered, evaporated on a steam-bath and the residue is dried at 100° and weighed.

Phenobarbitonum (B.P.). $C_{12}H_{12}O_3N_2 = 232.1$. M.p., 173° to 177° . Ash, not more than 0.05%. Phenobarbitalum, U.S.P. XII, yields not more than 0.15% ash.

Tabellæ Phenobarbitali (U.S.P. XII). Contain 94 to 106% of the labelled amount of $C_{12}H_{12}O_3N_2$, including all tolerances. In the assay, a weighed quantity of powdered tablets is shaken for 10 minutes with a reagent prepared by saturating a saturated solution of sodium chloride with barium hydroxide. After filtration, an aliquot of the filtrate is acidified with hydrochloric acid and extracted completely with chloroform-ether mixture (8:2). The combined extracts are washed with faintly acid water and the aqueous washings extracted with two further portions of solvent which are added to the main extract. The chloroform-ether solution is then filtered, the organic solvent removed, and the residue dried at 100° for 2 hours.

Phenobarbitonum Solubile (B.P.). $C_{12}H_{11}O_3N_2Na = 254.1$. Contains not less than 95% of the pure substance. Phenobarbitalum Sodicum, U.S.P. XII, on drying at 140° for 6 hours, loses not more than 7%. Contains 89 to 91.5% of phenobarbital, calculated on a moisture-free basis.

Tabellæ Phenobarbitali Sodici (U.S.P. XII). Contain 90 to 105% of the labelled amount of $C_{12}H_{11}O_3N_2Na$, including all tolerances. Assayed by the process described for phenobarbitone tablets.

Allobarbitonum (B.P.C.). $C_{10}H_{12}O_3N_2 = 208.1$. M.p., 171° to 172° . Ash, not more than 0.1%.

Phemitonum (B.P. Add. III). $C_{13}H_{14}O_3N_2 = 246.1$. M.p., 178° to 181° . Limit of neutral and basic substances, 0.25%.

Phenytoinum Solubile (B.P.C. Supp. III). $C_{15}H_{11}O_2N_2Na=274.1$. Assayed by direct titration of an alcoholic solution against N/10 hydrochloric acid, it contains not less than 98% of $C_{15}H_{11}O_2N_2Na$. Loss at 100° , not more than 1%. Diphenylhydantoin Sodicum, *U.S.P. XII*, loses not more than 2.5% at 100° and then contains 90.5 to 92% of $C_{15}H_{11}O_2N_2$. Assayed by extraction with ether from an acid solution, removal of the ether and weighing the base, dried to constant weight at 100° .

Identification of Barbiturates. A solution of 0.05 g. of the substance in 4 ml. of acetone is treated with 5 drops of Millon's reagent. A white precipitate dissolving on warming for 4 to 6 seconds then re-precipitated after a further 12 to 16 seconds warming indicates barbitone; a precipitate insoluble but becoming grey on warming for 10 to 15 seconds indicates allobarbitone. A white turbidity increasing on heating and shaking but dissolving with the simultaneous appearance of a slate-grey coloration after 15 to 25 seconds indicates phenylethyl-N-methylbarbituric acid (Prominal); solution of the white precipitate after heating for 8 to 10 seconds and appearance of a slate-grey precipitate after about a further 11 seconds indicates methylphenylbarbituric acid (Rutonal). A turbidity dissolving on shaking and warming with formation of a slate-grey precipitate in 20 to 30 seconds indicates Evipan. If no precipitate is formed with Millon's reagent and the acetone solution 0.05 g. of the substance is dissolved in 4 ml. of 95% alcohol and 5 drops of the reagent added. A yellowish-white precipitate becoming yellow on warming and white on the addition of 1 drop of hydrochloric acid, insoluble in 10 ml. of cold hydrochloric acid but soluble on warming indicates Evipan. A white precipitate insoluble on shaking or warming but soluble in 10 ml. of hot hydrochloric acid indicates phenobarbitone. A white turbidity insoluble on shaking or heating and giving a precipitate with 1 drop of hydrochloric acid but dissolving in 10 ml. of hot acid giving an amber-coloured solution indicates Phanodorm. A white turbidity soluble on warming and reacting with hydrochloric acid as Phanodorm but giving a colourless solution indicates butylethylbarbituric acid (Soneryl). No turbidity in the cold or on heating, but formation of a precipitate with 1 drop of hydrochloric acid, the precipitate dissolving with more acid indicates etho-2-butylethylbarbituric acid (Narcosol).—M. Paget and Tilly, *J. Pharm. Chim., Paris*, i/1937, 222.

Dissolve in concentrated sulphuric acid, add a few drops of formaldehyde solution and warm on a water-bath. Dial shows intense red colour with green fluorescence, Numal and Sandoptal give same reaction. Luminal, Rectoral, and Prominal give same red colour. Phanodorm gives yellow to orange. Veronal, Proponal, Soneryl, and Amytal give no reaction. If the formaldehyde is replaced by *p*-dimethylamino-benzaldehyde, Dial gives, on warming, a currant red colour, and addition of a few drops of water causes a change to reddish-violet. This is specific to Dial.—M. M. Pesetz, *J. Pharm. Chim., Paris*, 1938, 27, 247.

Methods of identification are given for barbiturates in solids, liquids, urine, blood and viscera.—M. M. Pesetz, *J. Pharm. Chim., Paris*, 1938, 28, 69.

Figures are given for the melting-points of numerous substituted barbituric acids together with their xanthidrol and *p*-nitrobenzyl derivatives.—J. C. Jespersen and K. T. Larsen, *Arch. Pharm., Berl.*, 1937, 28, per *Quart. J. Pharm.*, 1937, 544.

Determination of Phenobarbitone and Theobromine in Mixtures. The phenobarbitone is extracted from the mixture with alcohol-free ether. The residue of theobromine is boiled with dilute sulphuric acid, the solution cooled, made alkaline to phenol red with sodium hydroxide and then sulphuric acid carefully added until the solution is neutral. Then silver nitrate solution is added and the liberated nitric acid titrated with sodium hydroxide solution. The phenobarbitone is determined by extraction with alcohol-free ether, removing the ether, dissolving the residue in a mixture of alcohol water and sodium hydroxide, filtering and titrating an aliquot portion with silver nitrate solution. The method is applicable to tablets, providing stearates are absent. When the theobromine is present as salts, the method is slightly modified.—C. W. Bell, *J. Amer. pharm. Ass., Sci. Edn.*, 1941, 240.

Microchemical Detection of Barbituric Acid Compounds. The reactions of the following barbituric acid compounds are described in tabular form, and illustrated by drawings of the various types of crystals formed: Veronal (diethylbarbituric acid, m.p. 191°), Proponal (dipropylbarbituric acid, m.p. 145°), Dial (diallylbarbituric acid, m.p. 170° to 171°), Allonal (allylisopropylbarbituric acid, m.p. 141·5°), Soneryl (butylethylbarbituric acid, m.p. 123°), Luminal (phenylethylbarbituric acid, m.p. 174° to 176°), Rutonal (phenylmethylbarbituric acid, m.p. 226° to 228°), phanodorm (cyclohexenylethylbarbituric acid, m.p. 173°), and Sandoptal (isobutylallylbarbituric acid, m.p. 136° to 139°). When the 2% solutions of the compounds in potassium hydroxide are precipitated with nitric acid, Proponal, Dial, Luminal and Rutonal form well-defined crystals at once, but Veronal, Allonal, Soneryl, Phanodorm and Sandoptal give a finely divided amorphous precipitate, slowly becoming crystalline. On adding ammonium phosphate or other ammonium salt to the 2% solutions in potassium hydroxide, Veronal, Proponal, Dial, Luminal, Rutonal and Sandoptal give at once well-formed cubic and rhombic crystals, whilst after some time, Allonal, Soneryl and Phanodorm form crystals belonging to the binary system.

Reaction with thallium acetate. When the 2% potassium hydroxide solutions are treated with thallium acetate, only Veronal, Proponal, Allonal and Phanodorm give crystalline compounds. **Reaction with silver nitrate.** When the compounds are treated with 5% ammoniacal silver nitrate all but Veronal and Dial give soluble silver compounds. **Reaction with ammoniacal cuprous solution.** To the 2% potassium hydroxide solutions copper acetate is added and enough ammonia to dissolve the hydroxide precipitated. The reaction goes even better when Schweitzer's reagent is used instead. The copper ammonia compounds are all soluble except those of Dial, Rutonal and Sandoptal, which give amethyst-coloured crystals. **Reaction with bromine water.** All the compounds except Veronal give an amorphous precipitate with bromine water from the 2% solution in potassium hydroxide; with Proponal, Luminal, Rutonal and Phanodorm this slowly becomes crystalline. **Reaction with baryta water.** Only Dial gives crystals. From the above reactions it is possible to identify any of the given barbituric acid compounds.—L. van Itallie and A. J. Steenhauer, *Pharm. Weekbl.*, 1930, 977.

BARIUM

Barii Sulphas (B.P.). $\text{BaSO}_4 = 233\cdot4$. Loss at 110°, not more than 2%. Soluble barium compounds and sulphide, sulphite and thiosulphate are among the impurities tested for. The *U.S.P. XII* excludes barium sulphate which does not comply with a test for bulkiness of powder; on shaking 1 part by weight with water in a cylinder and adjusting to 10 volumes, shaking for 1 minute and standing 15 minutes the sediment measures not less than 2·4 volumes; acid-soluble substances, dried at 100° to 110° (in which no soluble barium salts are precipitated by sulphuric acid within 30 minutes), not more than 0·3%. Tests for sulphide, free acid or alkali, phosphate and heavy metals are included. The *P. Helv. V* gives the same bulkiness of powder test, but it should occupy not less than 3 volumes.

Baryta Sulphurata (B.P.C.). Contains not less than 60% of BaS. Assayed by titration with ammoniacal zinc solution, using alkaline lead indicator externally.

BELLADONNA

Belladonnæ Folium (B.P. Add. V). The leaves of *Atropa Belladonna*, Linn. or of *Atropa lutescens* Jacquem. (*Indian Belladonna*). The leaf of *Atropa Belladonna* contains not less

Belladonnæ Folii, N.F. VII, contains 0.27 to 0.33% *w/v* of alkaloids of belladonna leaf.

Extractum Belladonnæ Siccum (B.P.). Contains 0.95 to 1.05% of alkaloids of Belladonna leaf, calculated as hyoscyamine. **Extractum Belladonnæ, U.S.P. XII**, yields 1.15 to 1.35% of alkaloids. Assayed by the general method for hyoscyamus alkaloids. The powdered extract contains 1.25% of alkaloids of belladonna leaf, assayed similarly.

Assay of Extracts. The following method of assay of extract of belladonna is based on that of the German Pharmacopœia. 3 g. of the extract is dissolved in 5 ml. of warm water, and, after cooling and the addition of 2.0 g. of ammonia, shaken with 30 g. of ether. 1 g. of powdered tragacanth is added, and the mixture is strained through cotton wool. 20 g. of the ethereal solution is evaporated to dryness, the residue is dissolved in 2 ml. of alcohol, and titrated with N/100 hydrochloric acid, using methyl red as indicator. The method for belladonna leaf is as follows:—10 g. of powdered leaf is treated with 100 g. of ether and 7.0 g. of ammonia solution. After shaking at intervals during 30 minutes the liquid is strained off. The filtrate is mixed with 5 ml. of water, shaken well, allowed to stand, and filtered; 60 g. of the filtrate is evaporated to dryness and the residue dissolved in 10 ml. of ether. After adding 25 ml. of 0.25% hydrochloric acid and shaking, the ether is evaporated off and the liquid cooled and made up to 30 g. with 0.25% hydrochloric acid. The mixture is then shaken with 0.5 g. of talc and filtered, 25 g. of the filtrate being made alkaline with ammonia and shaken out three times with chloroform. The extract is filtered and evaporated to dryness, the residue is dissolved in 2 ml. of alcohol, 5 ml. of water is added, and the mixture is titrated with N/100 hydrochloric acid. For tincture of belladonna, 50 g. of the tincture is evaporated to 5 g. and treated with 50 g. of ether and 5 g. of ammonia. Further treatment is then as described above for the leaf, taking 40 g. of the ethereal solution.—A. Jermstad, *Norsk farm. Tidsskr.*, 1936, 44, 294.

Assay of Tablets. A satisfactory method is described to replace that in *U.S.P. XI* which is stated to be useless for tablets.—D. T. Wilson, *J. Amer. pharm. Ass.*, 1938, 27, 398.

Linimentum Belladonnæ (B.P.). Contains 0.350 to 0.400% *w/v* of alkaloids of Belladonna Root. Alcohol content 70 to 75% *v/v* of ethyl alcohol.

Tinctura Belladonnæ (B.P. Add. V). Contains 0.028 to 0.032% of alkaloids. **Tinctura Belladonnæ, U.S.P. XII**, contains 0.027 to 0.033% *w/v* of alkaloids of belladonna leaf.

Unguentum Belladonnæ, U.S.P. XII, yields 0.110 to 0.140% of alkaloids of belladonna leaf. Assayed by repeated extraction with chloroform from the ammoniacal liquid, completing by the general method for solanaceous alkaloids.

Atropina (B.P.). $C_{17}H_{23}O_3N = 289.2$. M.p., 114° to 116°. Ash, not more than 0.1%. Tests for limit of *l*-hyoscyamine and absence of apoatropine are included. The *U.S.P. XII* alkaloid is required to comply with the platonic chloride test for most other alkaloids and with tests for apoatropine and belladonnine.

An official method for the determination of atropine in tablets is described in *Methods of Analysis (A.O.A.C., 1940, 582)*. The atropine, extracted with chloroform from ammoniacal solution, is dissolved in a known excess of standard acid and back-titrated with standard sodium hydroxide, using methyl red as indicator.

Colorimetric Assay. A rapid method for the determination of atropine, hyoscyamine, and hyoscyne in hypodermic tablets or injections is described. The method depends upon a modification of Vitali's test and tables are given relating the red component of the colour obtained to the alkaloidal content. Details are also given for the determination of solanaceous alkaloids in the

presence of morphine which is removed by oxidation with 5% ferric chloride solution followed by extraction of the atropine in the usual manner after addition of sodium citrate and ammonia.—N. L. Allport and N. R. Jones, *Quart. J. Pharm.*, 1942, 240.

Atropinæ Sulphas (B.P. Add. I and VI).

$(C_{17}H_{23}O_3N)_2 \cdot H_2SO_4 \cdot H_2O = 694.5$. M.p., after powdering and drying at 135° for exactly 10 minutes, not lower than 188° . Loss at 136° , not more than 3%; ash, not more than 0.1%. A test for absence of apoeptropine is included. The salt is official in the *U.S.P. XII*; 0.2 g. in 5 ml. of sulphuric acid is required to produce no more colour than a specified control liquid. Atropinum sulphuricum, *P. Helv. V*, by titration of 5 ml. of standard solution and 3 ml. of chloroform with N/10 sodium hydroxide using phenolphthalein indicator, contains at least 99.3% of $(C_{17}H_{23}O_3N)_2 \cdot H_2SO_4$.

The melting-point is affected by the last minute trace of water which is not worth considering in the moisture determination, and it is therefore determined on the sample dried at 136° . When dried at this temperature the salt is comparatively stable in air.—T. A. Henry, *Pharm. J.*, i/1933, 86.

Tabellæ Atropinæ Sulphatis (U.S.P. XII). Tablets of 20 mg. or more contain 93 to 107% of the labelled amount of $(C_{17}H_{23}O_3N)_2 \cdot H_2SO_4 \cdot H_2O$, and tablets of less than 20 mg. contain 90 to 110% of the labelled amount, including all tolerances. For the assay a weighed quantity of powdered tablets is macerated with water and diluted sulphuric acid overnight. An aliquot of the filtrate is made alkaline with ammonia solution and extracted with chloroform. After removal of the chloroform the residue is dissolved in excess N/50 sulphuric acid and the solution titrated with N/50 sodium hydroxide, using methyl red indicator.

Homatropinæ Hydrobromidum (B.P.). $C_{16}H_{21}O_3N \cdot HBr = 356.1$. M.p. with partial decomposition, about 214° ; ash, not more than 0.1%. Homatropinæ Hydrobromidum, *U.S.P. XII*, melts at 212° and should also comply with a test for alkaloid precipitated with tannic acid, and a test for alkaloids giving the Vitali reaction. It is also required to give no precipitate with platinic chloride.

An official method for the determination of homatropine in tablets is described in *Methods of Analysis (A.O.A.C., 1940, 586)*. The homatropine, extracted with chloroform from ammoniacal solution, is dissolved in excess standard sulphuric acid and back-titrated with standard sodium hydroxide, using methyl red as indicator.

Eucatropinæ Hydrochloridum (U.S.P. XII). $C_{17}H_{21}O_3N \cdot HCl = 327.8$. Dried over sulphuric acid, contains 86 to 89% of the base, $C_{17}H_{21}O_3N$. Assayed by extraction with ether from an ammoniacal solution and drying the base at 80° . M.p., not below 83° ; ash, not more than 0.1%. (This substance was previously available in this country under the trade name of Euphthalmine—see Vol. I, 21st Edn.)

BISMUTHUM

Bismuthi Carbonas (B.P. Add. I). Residue of Bi_2O_3 on ignition, 90 to 92%. Limit tests for lead, copper, sulphate, alkalis and alkaline earths, nitrate and chloride are included and also a test for absence of silver. Bismuthi Subcarbonas, *U.S.P. XII*, after drying over sulphuric acid for 18 hours yields, on ignition, not less than 90% of Bi_2O_3 . Carbonate de Bismuth, *Fr. Cx*.

1937, yields 89 to 92% of Bi_2O_3 . Bismutum subcarbonicum, *P.G. VI*, contains 80.7 to 82.5% of bismuth. Bismutum subcarbonicum, *P. Helv. V*, corresponding approximately to the formula $(\text{BiO})_2\text{CO}_3 + \frac{1}{2}\text{H}_2\text{O}$ or $(\text{BiO})_2\text{CO}_3$, yields 90 to 92% of Bi_2O_3 . The residue of Bi_2O_3 obtained in the assay dissolved in hydrochloric acid should not produce a red coloration (selenium).

Tabellæ Bismuthi Subcarbonatis (N.F. VII). Yield Bi_2O_3 , not less than 83% and not more than 97% of the labelled amount of bismuth subcarbonate, including all tolerances. Assayed by heating to destroy organic matter, cooling and adding nitric acid, heating carefully and then igniting at a temperature below the fusion point of bismuth oxide. The residue is cooled, dissolved in nitric acid, the solution diluted to volume with 1% nitric acid and filtered. An aliquot of the filtrate is made alkaline with ammonia solution; the bismuth is precipitated with ammonium carbonate solution, heated on a steam-bath for 1 hour, collected on a tared Gooch crucible, washed and finally ignited to Bi_2O_3 .

A tentative method for the determination of bismuth compounds in tablets is described in *Methods of Analysis (A.O.A.C., 1940, 617)*.

Bismuthi Citras (B.P.C.). Residue of Bi_2O_3 , on ignition and re-ignition with nitric acid, not less than 55% and not more than 59%.

Liquor Bismuthi et Ammonii Citratis (B.P.C.). The following assay process is recommended as being more satisfactory than that of the *B.P.C.*: Dilute 10 ml. to 100 ml. To 25 ml. of the dilution add 50 ml. of water and sufficient nitric acid to produce a ppt. and to re-dissolve it. Add 25 ml. of water and strong solution of ammonia until a permanent ppt. is obtained, then add 2 ml. of nitric acid and heat to boiling. Add to the boiling solution a 10% solution of ammonium phosphate, at the rate of about 30 drops per minute, until all the bismuth is precipitated, then more quickly until 40 ml. in all has been added. Dilute to about 400 ml. with boiling water, heat on a water-bath for 15 minutes, filter through a Gooch crucible, wash by decantation three times and then on the filter with a 3% solution of ammonium nitrate containing a few drops of nitric acid, dry, ignite and weigh. 1 g. of BiPO_4 is equivalent to 0.7654 g. of Bi_2O_3 .—C. T. Bennett and N. R. Campbell, *Quart. J. Pharm.*, 1932, 515.

Bismuthi et Potassii Tartras (U.S.P. XII). By precipitation as phosphate contains the equivalent of 60 to 64% of Bi; alcohol-soluble extractive, not more than 0.5%.

Bismuthi et Sodii Tartras (B.P. Add. I). Contains 35 to 42% of Bi, determined by precipitation with ammonium phosphate and ignition. Complies with limit tests for lead and for copper.

Bismuthi et Sodii Tartras Acidus (B.P.C. Supp. V). By precipitation as sulphide, reprecipitation as carbonate, and ignition to oxide, it contains from 38 to 44% of Bi.

Bismuthi Hydroxidum. Corfield and Woodward were unable to substantiate the formula $\text{BiO} \cdot \text{OH}$ for a body made by the *Fr. Cx.* or other method. The only pure compound they could obtain was one of the formula $\text{Bi}(\text{OH})_3$.—*Pharm. J.*, i/1924, 83.

Bismuthi Oxyiodogallas (B.P.C.). Assayed for I content by solution in sodium hydroxide, boiling with N/10 silver nitrate

and nitric acid and back titration with N/10 ammonium thiocyanate, it contains not less than 20%. Residue of Bi_2O_3 , 43 to 45%. Bismutum oxyiodogallicum, *P. Helv. V*, should contain 20 to 24.5% of iodine and yield from 45 to 48.5% of Bi_2O_3 . Iodine is determined by boiling with N/10 silver nitrate and nitric acid until nitrous vapours cease to be evolved and the silver iodide is yellow; the diluted solution is then treated with potassium permanganate until a violet colour persists 1 minute, decolorised with ferrous sulphate and titrated with ammonium thiocyanate. Bi determined by treatment with nitric acid, evaporation and ignition.

Bismuthi Salicylas (B.P.). Residue on ignition and re-ignition, not less than 62% and not more than 66% of Bi_2O_3 . A limit of free salicylic acid of 0.1% is included. Bismuthi Subsalicylas, *U.S.P. XII*, after drying to constant weight at 100°, yields 62 to 66% of Bi_2O_3 . Bismutum subsalicylicum, *P.G. VI*, contains 56.5 to 58.5% of bismuth. The *Fr. Cx.* 1937 requires the same Bi_2O_3 content as the *U.S.P. XII*. Bismutum subsalicylicum, *P. Helv. V*, corresponds approximately to the formula $\text{C}_6\text{H}_4(\text{OH})\text{COOBiO}$ and yields 63 to 65.2% of Bi_2O_3 .

Bismuthi Oxychloridum (B.P. Add. I) determined by precipitation as phosphate and ignition, has a Bi content of 79 to 81%, and a Cl content of not less than 12.5% by titration with silver nitrate and ammonium thiocyanate. Limit tests for lead, copper and sulphates are included and a nitrates limit:—Add 5 ml. of indigo carmine solution and 15 ml. of nitrogen-free sulphuric acid in 2 portions to 0.5 g. in 10 ml. of water, boil and set aside for 1 minute; the blue colour should not be entirely discharged.

Bismuthi Subgallas (B.P. Add. IV). Loses not more than 5% at 100°, and then yields by re-ignition with nitric acid from 52 to 57% of Bi_2O_3 . Limit of free gallic acid, by solubility in alcohol (90%), not more than 0.25%. The *N.F. VII* salt has the same standard on the substance dried at 100° for 3 hours. Gallate de Bismuth, *Fr. Cx.* 1937, yields not less than 52% Bi_2O_3 . Bismutum subgallicum, *P. Helv. V*, yields 52 to 56.5% of Bi_2O_3 .

Tabellæ Bismuthi Subgallatis (N.F. VII). Yield Bi_2O_3 , not less than 48% and not more than 61% of the labelled amount of bismuth subgallate, including all tolerances. Assayed by the process described for bismuth subcarbonate tablets.

Bismutum bitannicum (P. Helv. V). By ignition, evaporation with nitric acid and re-ignition, contains 20 to 24% of Bi_2O_3 . Free tannic acid, by agitation for 2 minutes with 10 volumes of alcohol, filtration and drying at 103° to 105°, not more than 1.2%.

Bismuthi Subnitras (B.P.C. Add. V). Residue of Bi_2O_3 , not less than 79% and not more than 81%. Complies with tests for absence of silver and limits of chloride, sulphate, alkalis and alkaline earths, lead, and copper. Dissolves completely without effervescence in an equal quantity of warm nitric acid. Bismuthi Subnitras, *U.S.P. XII*, yields not less than 79% of bismuth oxide

after drying over sulphuric acid. Azotate Basique de Bismuth Lourd, *Fr. Cx.* 1937, yields 79 to 81% of Bi_2O_3 and contains 15.5 to 17% of N_2O_5 , determined by treatment with ferrous sulphate solution and back titration with N/10 potassium permanganate. Azotate Basique de Bismuth Léger, *Fr. Cx.* 1937, yields 82 to 89% of Bi_2O_3 . Bismutum subnitricum, *P.G. VI*, contains 70.9 to 73.6% of bismuth. Bismutum subnitricum, *P. Helv. V*, contains 79 to 82% of bismuth calculated as Bi_2O_3 and is stated to correspond approximately to the formula $\text{Bi}(\text{OH})_2\text{NO}_3 + \text{OBiNO}_3 + \text{OBiOH}$ or $2\text{Bi}(\text{OH})_2\text{NO}_3 + \text{OBiOH}$.

The proportion of N_2O_5 in this and other nitrates may be determined by the following method of the Board of Agriculture and Fisheries (Leaflet No. 18, p. 116):—

To 0.5 g. in a 500 ml. Erlenmeyer flask add 50 ml. of water and 20 ml. of sulphuric acid (sp. gr., 1.35) followed by 10 g. of reduced iron. Close the flask with a rubber cork through which passes a thistle funnel the head of which is half filled with glass beads, boil for five minutes and wash back into the flask any liquid among the beads. Boil for three minutes, then distil with 50 ml. of 50% sodium hydroxide, collecting the ammonia evolved in 50 ml. of N/10 sulphuric acid, and back titrate the excess acid with N/5 alkali. Conduct a blank experiment under the same conditions.—C. E. Corfield and G. R. A. Short, *Yearb. Pharm.*, 1924, 573.

Dragendorff's Test for alkaloids. Bismuth subnitrate 8, nitric acid, sp. gr. 1.18, 20; add this solution gradually to a concentrated solution of potassium iodide, 22.7. Cool, decant from potassium nitrate formed, and dilute to 100 with water. The solution precipitates most alkaloids.

A suggested modification:—Dissolve bismuth carbonate 64 in hydrochloric acid 85 and add water 500 containing potassium iodide 166. Finally make up with water to 800. This eliminates nitric acid which causes decomposition, and the proportion of potassium iodide is less. With this formula there is no trouble with crystals of potassium nitrate.

Thresh's Reagent. Bismuth citrate 2.4 g., water 20 ml., ammonia q.s., made up to 30 ml. with water and added to a solution of potassium iodide 2 g. in nitric acid 45 ml. Is similar in use to above.

Tabellæ Bismuthi Subnitratis (N.F. VII). Yield Bi_2O_3 , not less than 73% and not more than 85% of the labelled amount of bismuth subnitrate, including all tolerances. Assayed by the process described for bismuth subcarbonate tablets.

Bismuthi Tribromphenas (B.P.C. Supp. IV). Assayed by precipitation, as for the subchloride, after decomposition with sodium hydroxide and dissolving the precipitate in nitric acid, it contains not less than 47% and not more than 49.5% of Bi. A limit of free tribromophenol, by addition of excess sodium hydroxide to the alcohol-soluble matter and titration with standard hydrochloric acid, is equivalent to about 3%. Bismutum tribromphenylicum, *P.G. VI*, should contain not less than 44.9% of bismuth. Bismutum tribromphenylicum, *P. Helv. V*, yields from 50 to 55% of Bi_2O_3 .

Bismuthum Præcipitatum (B.P. Add. I). Determined by precipitation as phosphate, and ignition, it contains not less than 98.5% of metallic bismuth. In the limit test for silver the substance is dissolved in hydrochloric acid with potassium chlorate, evaporated to remove chlorine, diluted with hydrochloric acid and tested with potassium iodide solution. A limit test for copper is included.

Detection of Silver in Bismuth Salts.

Dissolve 0.02 g. of bismuth carbonate in 2 ml. of dilute hydrochloric acid; add 2 ml. of 0.03% solution of *p*-dimethylaminobenzylidene-rhodanin and 1.8 ml. of distilled water. Add a few drops of water if necessary to make the liquid turbid. In the presence of 1 in 40,000 of silver the precipitate is red to violet. For bismuth salicylate, heat to boiling 0.5 g. with 5 ml. of dilute hydrochloric acid, shaking the tube. To 2 ml. of filtered liquid add 2 ml. of the above reagent and 3.8 ml. of water, or more if necessary, to produce turbidity. In the presence of 0.002% of Ag the ppt. is coloured violet.—J. F. Reith, *Pharm. J.*, i/1933, 316.

Sensitive Colour Reactions for Bismuth.

To a suitable volume of the aqueous solution to be tested, e.g. 10 ml., add 2 ml. of dilute hydrochloric acid and 0.5 g. of potassium iodide. Mix, and add 5 ml. of acetone or industrial spirit and 5 ml. to 10 ml. of ethyl acetate. Shake and allow to separate; a red coloration in the upper layer indicates bismuth. By suitable modification the test can be applied quantitatively and is suitable for the rapid determination of bismuth in urine and animal tissues.—A. D. Powell, *Quart. J. Pharm.*, 1933, 465.

Traces of bismuth in the presence of other metals can be separated by extraction, after preliminary treatment, with a solution of diphenylthiocarbazon in chloroform. After further treatment the bismuth compound is extracted with a mixture of 3 parts of amyl alcohol and one part of ethyl acetate. The colour of the solution is compared with those obtained from known amounts of bismuth.—L. A. Haddock, *Analyst*, 1934, 163.

Quinine Iodobismuthate. It should pass the following tests. It should dissolve completely in acetone containing a little water, and 0.25 g. shaken with 5 ml. of chloroform should not impart a violet colour to the latter (free iodine). It should not lose more than 1% when dried at 100°. The three components can be estimated by dissolving the substance in a little acetone, adding silver nitrate, which precipitates the iodine and the bismuth, dissolving the bismuth from the precipitate with nitric acid, leaving the silver iodide. The quinine may be determined polarimetrically in the first filtrate. The author suggests the following standards: bismuth 24.30%, quinine 17.50%, iodine 58.0%.—G. Vita and L. Bracaloni, per *Quart. J. Pharm.*, 1937, 81.

Assay. Dissolve 1 g. of sample in 10 ml. of acetone, add 0.09 g. of silver nitrate in 20 ml. of water, and heat to drive off the acetone. Then add 50 ml. of alcohol and allow the precipitate to settle. Pour off the clear liquid containing quinine nitrate through a Gooch crucible, wash the precipitate with warm alcohol by decantation, pouring the washings through the Gooch crucible, evaporate the filtrate and washings to small bulk, add 4 ml. of N/1 sulphuric acid and dilute to 50 ml. Determine the quinine in this solution polarimetrically. The precipitate of silver iodide and basic bismuth nitrate is washed with a mixture of nitric acid and water, equal parts, and transferred to the crucible with more dilute nitric acid. It is then washed, dried, and weighed. The dilute nitric acid filtrate is treated with excess of ammonium carbonate, the liquid boiled, and the precipitate washed, dried, ignited and weighed as Bi_2O_3 .—N. Bracaloni, *J. Pharm. Chim., Paris*, ii/1935, 49.

The quinine is determined by adding sodium hydroxide to an aqueous solution of the compound and extracting the precipitated alkaloid with chloroform, evaporating the latter and weighing. The aqueous solution is acidified with nitric acid and the liberated iodine extracted with chloroform. The solution of iodine in the chloroform is diluted with alcohol, 1 or 2 g. of sodium bicarbonate added and the iodine titrated with N/10 sodium thio-sulphate. The residual aqueous solution is boiled to remove the chloroform, neutralised with sodium hydroxide, made slightly acid and saturated with hydrogen sulphide to precipitate the bismuth. The latter is collected, washed with hydrogen sulphide water, alcohol, ether and carbon disulphide and weighed.—G. N. Thomis and G. P. Kopanaris, *J. Pharm. Chim., Paris*, 1939, 30, 193.

Determination of Bismuth. About 1 g. is mixed in a warm 150 ml. beaker with 10 ml. of acetone and stirred with a mixture of 5 ml. of 20% tartaric acid solution and 5 ml. of 30% sodium hydroxide solution free from halogen. When the solution is decolorised, 20 ml. of water is added, the mixture heated for 30 minutes on a boiling water-bath, cooled and filtered, the beaker being rinsed with a total of 50 ml. of water. 125 ml. of 16% ammonium carbonate

solution is added, the mixture heated on a boiling water-bath for 4 hours and then allowed to stand for 24. The precipitated bismuth carbonate is washed by decantation, filtered, dried, ignited, and weighed as Bi_2O_3 .—M. François and L. Seguin, *J. Pharm. Chim., Paris*, i/1937, 341.

Determination of Bismuth in Biological Material. The material is digested with sulphuric and perchloric acids in a Kjeldahl flask until all the organic material is destroyed and the excess of perchloric acid has been driven off. The digest is diluted with water, 20% sodium citrate solution added and the mixture made alkaline to pH 8 with ammonia. A 2% solution of sodium diethyldithiocarbamate is added and the mixture extracted three times with ether. The combined ethereal extracts are evaporated to dryness in a Kjeldahl flask, and the organic material destroyed by heating with 1 ml. of concentrated sulphuric acid and 1 ml. of perchloric acid. After cooling, the mixture is diluted with water and a 10% thiourea solution added. The colour of the solution is compared with a standard made from a solution of bismuth in 20% sulphuric acid mixed with 5% thiourea solution.—S. L. Tompsett, *Analyst*, 1938, 250.

BUCHU

Buchu (*B.P. Add. I*). The dried leaves of *Barosma betulina* (Thunb.) Bartl. and Wendl. only. Contains not more than 5% of its stem. Other foreign organic matter, not more than 2%. Ash, not more than 5%. Yields not less than 20% of alcohol (25%) soluble extractive. The *N.F. VII* allows the dried leaf of *Barosma betulina* (Thunberg) Bartling et Wendland (Short Buchu) or of *Barosma crenulata* (Linné) Hooker (oval buchu) or of *Barosma serratifolia* (Curtis) Willdenow (long buchu). It should contain not more than 8% of its stems, not more than 2% of other foreign organic matter, not more than 1% of acid-insoluble ash, and not less than 1.25% *v/w* of volatile oil determined by the method prescribed.

An account of the histology of buchu and of the leaves of other species of *Barosma*. A key is given for the identification of the powdered leaves of various species of *Barosma*.—T. E. Wallis and T. Dewar, *Quart. J. Pharm.*, 1933, 347.

Uva Ursi (*B.P.C.*). Contains not more than 5% of foreign organic matter.

Determination of Arbutin. 0.5 to 2.5 g. of bearberry is macerated in boiling water for one hour. The liquid is filtered, the residue washed and the mixed filtrate and washings rendered alkaline with ammonia. 20 ml. of filtered 10% lead acetate solution is added, the mixture heated on a water-bath for 15 minutes, then allowed to stand 2 hours and filtered through a Buchner filter. The filtrate is freed from lead by means of hydrogen sulphide and again filtered, the product being boiled to remove excess of hydrogen sulphide. The solution is made alkaline with ammonia, 1 ml. of 10% phosphomolybdic acid solution is added and then water to 500 ml. The colour produced is tested against known quantities of a standard arbutin solution treated similarly with phosphomolybdic acid. The process is applied to a liquid extract by diluting 1 to 5 ml. to 200 ml., filtering and washing, and treating the liquid thus obtained as described above for the infusion of the crude drug.—J. E. Ball and C. O. Lee, *J. Amer. pharm. Ass.*, 1937, 698.

CAFFEINA

Caffeina (*B.P.*). $\text{C}_8\text{H}_{10}\text{O}_2\text{N}_4\cdot\text{H}_2\text{O}=212.1$. M.p., after drying at 100° , 235° to 237° . Loss at 105° , not more than 8.5%. Ash, not more than 0.1%. Caffeina, *U.S.P. XII*, should lose not more

than 9% of its weight at 80°. Ash limit, 0.1%. Tests for carbonisable substances and heavy metals are included.

Caffeine and theobromine give no precipitate with Mayer's reagent, distinguishing them from the majority of alkaloids. Tea contains a minimum of 3.5% of caffeine and a maximum of 4.0%. Raw coffee, about 1.2%, and when roasted, about 1.3%. For manufacture, tea dust with the largest yield of alkaloid is extracted.

Tea. When there is neither caffeine nor tannin present in quantity exceeding that which the compound of them (caffeine tannate) contains, the tea is pronounced by the taster as of good quality. Caffeine and tannin occur mostly (in good teas) in the ratio of 1:3, which is virtually the ratio in caffeine tannate. Cold water extracts only a very small proportion of the total caffeine in tea, though solubility is 1.35% at 16°. Caffeine is taken up always as tannate.

The Food and Drug Administration of the U.S. Dept. of Agriculture defined tea as the tender leaves, leaf buds, and tender internodes of different varieties of *Thea sinensis* L., prepared and cured by recognised methods of manufacture; conforms in variety and place of production to the name it bears; ash, 4 to 7%.—S.R.A., F.D., No. 2, Rev. 5, Nov. 1936.

Coffee. The caffeine in tea, being in the form of caffeine tannate, is precipitated by the gastric juice and, therefore, the caffeine is probably not absorbed until it reaches the alkaline alimentary tract. In the case of coffee, however, in whatever form the caffeine may be present it is soluble in both alkaline and acid fluids and, therefore, the absorption in this case is probably in the stomach, hence the more prompt action as restorative.

Extracts purporting to be pure coffee are comparatively rare, coffee and chicory extracts being the commoner. But judging from samples received the caffeine in the two classes does not differ much, the coffee extracts (12 in number) containing from 0.1 to 0.33% of caffeine (average 0.21), and the 45 coffee and chicory extracts containing from 0.05 to 0.38% (average 0.19), though there might be some doubt about the designation of some of the coffee extracts. A dry extract examined contained 6.8% of caffeine. Taking the caffeine as a basis of evaluation, the poorest extracts gave a "coffee" only of from one-tenth to one-fifth the strength of ordinary breakfast coffee made from the berry, and even at the best they were but a poor approach to the real article.—E. Hinks, *Lancet*, i/1936, 387.

Decaffeinated Coffee. The amount of caffeine removal in current specimens approximates well over 90%, assuming that 1.2% of caffeine by weight was originally present.—*J. Amer. med. Ass.*, ii/1928, 883.

The Food and Drug Administration of the U.S. Dept. of Agriculture defined coffee as the seed of cultivated varieties of *Coffea arabica*, *C. liberica*, and *C. robusta*. Green coffee, raw coffee, unroasted coffee—coffee with only a small portion of its spermoderm, conforming in variety and place of production to the name it bears. Roasted coffee, ("coffee") properly cleaned green coffee which has become brown by roasting and has developed its characteristic aroma.—S.R.A., F.D. No. 2, Rev. 5, Nov. 1936.

Caffeina Citras (B.P.C.). $C_8H_{10}O_2N_4 \cdot C_6H_5O_7 = 386.2$. By extraction with chloroform from a solution made alkaline with sodium hydroxide and drying at 100°, the anhydrous caffeine obtained is from 46 to 51%. Loss at 80°, not more than 5%. Ash, not more than 0.1%. Caffeina Citrata, U.S.P. XII, loses not more than 5% at 80° and then contains from 48 to 52% of anhydrous caffeine. Ash limit, 0.1%. Caffeinum citricum, *P. Helv. V*, contains not more than 2% of moisture and from 48.8 to 50.8% of anhydrous caffeine. **Assay:** Dissolve 0.5 g. in 2.5 ml. of water; add, after cooling, 25 g. of chloroform and 2 g. of 30% solution of sodium hydroxide, and shake for 5 minutes; add 0.5 g. of powdered tragacanth and shake again. After 5 minutes, filter and distil off the chloroform from 20 g. of the filtrate in a tared flask; dry the residue for 30 minutes at 103°

to 105° and weigh, adding 0.0017 g. of caffeine to the weight obtained. The result gives the weight of anhydrous caffeine in 0.4 g. of the caffeine citrate.

Tabellæ Caffeinæ Citrati (N.F. VII). Contain $C_8H_{10}O_2N_4$, not less than 43% and not more than 53% of the labelled amount of citrated caffeine, including all tolerances. Assayed by dissolving as completely as possible in water, making alkaline with sodium hydroxide solution, extracting with chloroform, filtering each chloroform extract, removing the chloroform and drying the residue at 80°.

Caffeina et Sodii Benzoas (B.P.). The dried substance contains 47 to 50% of $C_8H_{10}O_2N_4$ and 50 to 53% of $C_7H_5O_2Na$. Loss at 105°, not more than 5%. Caffeine is determined as in Caffeinæ Citras; benzoic acid is extracted with ether from the aqueous liquids and washings from the caffeine extraction made acid with dilute sulphuric acid; after evaporation of the ether, the residue is titrated in alcoholic solution with N/10 sodium hydroxide to phenol red and calculated to the sodium compound. M.p. of the separated caffeine, 235° to 237°, and of the separated benzoic acid, 121° to 122°. Caffeina et Sodii Benzoas, U.S.P. XII, loses not more than 3% at 80° and then contains 47 to 50% of anhydrous caffeine and 50 to 53% of sodium benzoate. In the assay, the caffeine is extracted with chloroform from solution made just alkaline to phenolphthalein with N/10 sodium hydroxide, drying the separated caffeine at 80°; the liquid from which the caffeine has been separated is mixed with ether and the benzoic acid titrated with N/10 hydrochloric acid to methyl orange indicator. Coffeinum-Natrium benzoicum, P.G. VI, contains not less than 38% of anhydrous caffeine.

Tabellæ Caffeinæ cum Sodii Benzoate (N.F. VII). Yield $C_8H_{10}O_2N_4$ not less than 43.5% and not more than 53.5% of the labelled amount of caffeine with sodium benzoate, including all tolerances. Assayed by the process described for citrated caffeine tablets.

Caffeina et Sodii Salicylas (B.P.C.). Assayed as Caffeina et Sodii Benzoas, it contains 47 to 50% of anhydrous caffeine, and 50 to 53% of sodium salicylate, calculated with reference to the substance dried at 105°. Loss at 105°, not more than 5%. Caffeina cum Sodii Salicylate, N.F. VII, contains 48 to 52% of anhydrous caffeine and 48 to 52% of sodium salicylate, after drying at 80°. After extraction of the caffeine with chloroform from a solution made alkaline with sodium hydroxide, the salicylate is determined by treatment during 30 minutes with N/10 bromine and hydrochloric acid, and the iodine liberated from potassium iodide titrated with standard thiosulphate. Coffeinum-Natrium Salicylicum, P.G. VI, should yield not less than 40% of anhydrous caffeine. It is assayed as follows:—

Dissolve 0.5 g. of caffeine sodium salicylate in 1 ml. of water in a 50 ml. measure. Add to the solution 25 g. of chloroform and 2.5 g. of sodium hydroxide solution. Shake thoroughly for 5 minutes. After adding 0.3 g. of tragacanth, shake again for several minutes, and after a further 5 minutes pour 20 g. of the chloroform solution (equivalent to 0.4 g. of the sample) through a little

wool into a weighed flask. On evaporating the chloroform and drying the residue at 100° , the residue must be at least 0.16 g., representing 40% of caffeine.

Caffeinae Hydrobromidum (B.P.C.). $C_8H_{10}O_2N_4 \cdot HBr \cdot 2H_2O$ = 311.1. Yields from 60 to 63% of anhydrous caffeine. Ash, not more than 0.1%.

Guarana (B.P.C.). Contains not less than 3.5% of caffeine, calculated as anhydrous. Assayed by maceration with chloroform and ammonia, evaporation of an aliquot part of the filtered solution, solution of the residue in warm N/5 sulphuric acid and extraction of the filtered liquid, made alkaline with sodium hydroxide, with chloroform, finally evaporating and drying at 100° . Guarana, *N.F. VII*, yields not less than 4% of caffeine, by a similar extraction; acid-insoluble ash limit, 0.5%.

Theobromina (B.P.C.). $C_7H_8O_2N_4$ = 180.1. Loss at 100° , not more than 3%. Ash, not more than 0.1%. A caffeine limit, by extraction with chloroform from sodium hydroxide solution, is equivalent to 1%.

A method for the determination of theobromine in tablet mixtures. The method consists in the gravimetric precipitation of the insoluble compound formed by the action of phosphato-dodecatungstic acid on hot strongly acidified solutions of the tablet mixture. The precipitate is filtered while hot, washed, dried and weighed.—A. G. Richardson and Y. C. Campbell, *J. Amer. pharm. Ass., Sci. Edn.*, 1942, 24.

The second report of the Commission Internationale des Spécialités includes an agreed method for the determination of theobromine in tablets or other preparations containing salicylates in which by the addition of silver nitrate, a theobromine-silver compound is formed and the liberated nitric acid is titrated with alkali to phenol red.—*Bull. Féd. int. Pharm.*, 1938, 89.

Tentative methods for the determination of theobromine in theobromine calcium, and in tablets of theobromine calcium are described in *Methods of Analysis (A.O.A.C., 1940, 593)*.

Cocoa. The ground nibs of *Theobroma Cacao* from which most of the fat has been removed. Cocoa is sometimes treated with an alkali or an alkaline salt, such as potassium carbonate, to render it "soluble," the alkali probably producing a more perfect emulsion of the fat. Before and after treatment with alkali, cocoa shows essentially the same lack of solubility, and hence the designation "soluble cocoa" is misleading and deceptive.

In pure untreated cocoa, the ash should generally be below 5.5%, with an alkalinity not exceeding 3.75; while with cocoas treated with alkali the ash is often as high as 8.5%, with an alkalinity exceeding 6. (Azor Thurston, *Pharmaceutical and Food Analysis*, 1923.) A number of samples were examined in 1925 by W. H. Martindale, and it was found that in several cases the high ash and alkalinity were suggestive of the use of alkali in their preparation. The % ash varied from 2.8 to 7.5, the average being 5.7. The alkalinity varied from 3.5 to 11.0, the average volume of N/10 acid required to neutralise 1 g. being 7.5 ml. None of the samples contained added starch. When treated with water as directed, the samples with high alkalinity did not give a better suspension than the untreated samples. The examination of a number of cocoas on the market showed moisture to range from 3 to 8%. Nitrogenous matter (N \times 6.3) 19 to 20%, fat 26 to 31%, mineral matter 3.9 to 8.8%, theobromine 1.7 to 2%.

Cacao (N.F. VII). Non-volatile ether-soluble extractive, not more than 22%. The dried ether-insoluble residue yields not more than 7% of crude fibre, ash not more than 8% and acid-insoluble ash not more than 0.4%.

Theobromina et Sodii Salicylas (B.P.). Loss at 110° , not more than 5%, and then contains not less than 46% of $C_7H_8O_2N_4$,

not less than 41% of $C_7H_5O_3Na$, and not more than 6.9% of Na additional to that contained in the sodium salicylate. Assayed by the processes of the *B.P.* '32; for theobromine, about 1 g. in 10 ml. of water is shaken with 2 ml. of N/1 sodium hydroxide and 0.6 ml. of dimethyl sulphate for five minutes and set aside, with frequent shaking, for thirty minutes; a further 3 millilitres of N/1 sodium hydroxide is added and shaken well for one or two minutes, and the caffeine extracted immediately with successive portions of chloroform; the washed mixed chloroform solutions are evaporated and dried at 100° for 1 hour; 1 g. of the anhydrous caffeine obtained is equivalent to 0.9278 g. of $C_7H_5O_2N_4$. Salicylic acid is determined on a solution, previously acidified with dilute hydrochloric acid, from which the theobromine has been precipitated with ammonia, the mixture being allowed to stand for 3 hours, then filtered and the filtrate acidified, by extraction with ether, finally titrating in alcoholic solution with N/10 sodium hydroxide to phenol red. Additional sodium is titrated in warm aqueous solution with N/1 hydrochloric acid to phenol red. A limit test of caffeine of 0.5% is included.

The *B.P.* method can give accurate results and a clean, pure caffeine, but some workers obtain an acid residue which contains sulphate and melts at a temperature much below that of pure caffeine. It is essential for correct results to obtain a clean water-free chloroform solution before evaporation; this can be done by warming and pouring into a clean beaker or by filtering. With old samples of theobromine and sodium salicylate the volume of N/1 NaOH in the initial stage can with advantage be increased to 4 ml. and a further 10 ml. of H_2O added with the second quantity of N/1 NaOH. With more water, however, care must be taken to see that the caffeine is extracted completely. Theobromina cum Sodii Salicylate, *N.F. VII*, should yield not less than 46.5% of theobromine and not less than 35% of salicylic acid, after drying at 110° , at which temperature the loss should be not more than 10%. 2 g. of the dried substance requires not more than 5.5 ml. of N/1 hydrochloric acid to neutralise to phenolphthalein; the theobromine is filtered from this neutralised solution, made just alkaline to litmus with ammonia, and dried at 100° , a correction being added for the solubility in liquid and washings; salicylic acid is extracted from these with chloroform. Theobromino-natrium salicylicum, *P.G. VI*, contains not less than 40% of theobromine; assayed by neutralisation with N/10 hydrochloric acid to methyl red and after standing at room temperature for 3 hours, collecting the precipitated theobromine and drying at 100° . Theobromino-natrium salicylicum, *P. Helv. V*, contains not more than 5% of moisture, 10.5 to 10.7% of NaOH, and from 46.5 to 48% of theobromine. Assay for theobromine:—To a solution of 1 g. in 5 ml. of boiling water, a boiling solution of 0.5 g. of boric acid in 10 ml. of water is added, the mixture boiled and cooled till theobromine separates. Collect the precipitate after 3 hours, wash twice with 5 ml. of water, dry at 103° to 105° and weigh.

Theophyllina (*B.P. Add. I*). $C_7H_8O_2N_4 \cdot H_2O = 198.1$. M.p., 269° to 272° . Loss at 100° , not more than 9.5%. Ash, not more than 0.1%. The *U.S.P. XII* allows the same moisture limit for Theophyllina; 0.2 g. complies with the specified test for carbonisable substances.

Tabellæ Theophyllinæ (*U.S.P. XII*). Contain 94 to 106% of the labelled amount of $C_7H_8O_2N_4 \cdot H_2O$, including all tolerances. Assayed by disintegrating 20 tablets in water, adding ammonia solution and making up to volume with water, filtering and adding N/10 silver nitrate to an aliquot of the filtrate, allowing to stand 1 hour, filtering and washing the residue with water, acidifying the filtrate with nitric acid and finally titrating the excess N/10 silver nitrate with N/10 ammonium thiocyanate.

A tentative method for the determination of theophylline in solutions and tablets is described in *Methods of Analysis* (*A.O.A.C.*, 1940, 593).

Theophyllina et Sodii Acetas (*B.P.*). By treatment with dimethyl sulphate and sodium hydroxide, followed by extraction with chloroform, it yields caffeine equivalent to not less than 55% of anhydrous theophylline. Theophyllinæ et Sodii Acetatis, *U.S.P. XII*, contains 55 to 65% of anhydrous theophylline. Theophyllino-natrium aceticum, *P. Helv. V*, contains from 3 to 4% of moisture, equivalent to 1 molecule of water of crystallisation, and about 65% of theophylline, but no assay process for the latter is prescribed.

In the assay processes for Theophyllina et Sodii Acetas and Theobromina et Sodii Salicylas, the dimethyl sulphate must be in slight excess at the end of the reaction, and the caffeine must be shaken out rapidly after the addition of the sodium hydroxide, since it is quickly decomposed by strong alkalis.—*P. A. W. Self, Pharm. J.*, ii/1933, 244.

Theophylline may be determined in the presence of sodium acetate by van Giffen's modification of Boie's method, if bromocresol purple is used as indicator instead of phenol red.—*G. J. W. Ferrey, Quart. J. Pharm.*, 1940, 274.

Tabellæ Theophyllinæ et Sodii Acetatis (*U.S.P. XII*). Contain anhydrous theophylline, $C_7H_8N_4O_2$, corresponding to 53 to 67% of the labelled amount of theophylline and sodium acetate, including all tolerances. Assayed by the process described for theophylline tablets.

Theophyllina Æthylenediaminica (*U.S.P. XII*). Contains 75 to 82% of anhydrous theophylline ($C_7H_8O_2N_4$) and 12.3 to 13.8% of ethylenediamine ($C_2H_4(NH_2)_2$). Assayed for theophylline by the following process:—0.25 g. is dissolved on a steam-bath in 50 ml. of water with 8 ml. of ammonia solution. Excess N/10 silver nitrate is added and the warming continued for 15 minutes. The precipitate is filtered with the aid of a pump and washed with three portions of water. The excess silver nitrate in the cooled and acidified filtrate and washings is then titrated with N/10 ammonium thiocyanate, using ferric ammonium sulphate indicator; 1 ml. of N/10 silver nitrate is equivalent to 0.0180 g. of $C_7H_8O_2N_4$. The ethylenediamine is determined by titration with N/10 hydrochloric acid to methyl orange; 1 ml. N/10 acid is equivalent to 0.003004 g. of $C_2H_4(NH_2)_2$.

Tabellæ Theophyllinæ Æthylenediaminica (*U.S.P. XII*). Contain anhydrous theophylline, $C_7H_8N_4O_2$, corresponding to 73 to 84% of the labelled amount of theophylline ethylenediamine, including all tolerances. Assayed by the process described for theophylline tablets.

Reimer's Process. Dissolve 0.3 g. of aminophylline in 5 ml. of N/2 sodium hydroxide in a separator. Make acid to litmus paper with N/2 hydrochloric acid, then add 5 ml. more and shake for 1 minute with 25 ml. of a mixture of chloroform and isopropyl alcohol (3+1). Allow to settle for at least 5 minutes and draw off the lower layer into a second separator containing 10 ml. of water acidified with N/2 hydrochloric acid. Shake well, allow to settle for at least 5 minutes, and filter off the solvent into a tared flask through a pledget of cotton wool. Repeat the extraction with 5 more portions, each of 20 ml., of the solvent mixture, washing each time in the second separator. Evaporate the solvent,

add 2 ml. of absolute ether, evaporate, dry at 80°, and weigh the residue of anhydrous theophylline.—L. E. Warren, *J. Ass. off. agric. Chem., Wash.*, 1937, 580. See also F. Reimers, *ibid.*, 632.

The Self-Rankin process applied to aminophylline gives results that are about 3% low and occasionally the results are erratic. Possibly the method is not so satisfactory for theophylline as for theobromine. For aminophylline the Reimer's process is the most satisfactory of any tried, the results being 0.25 to 0.6% high.—L. E. Warren, *J. Ass. off. agric. Chem., Wash.*, 1937, 587.

CALCIUM

Calcii Carbonas (B.P.). $\text{CaCO}_3 = 100.1$. Loses at 100°, not more than 1% and then contains not less than 98.5% of CaCO_3 . **Calcii Carbonas Præcipitatus, U.S.P. XII**, after drying at 200°, contains not less than 98.5% of CaCO_3 and is assayed by precipitation with excess ammonium oxalate in ammoniacal solution and titration of the acidified precipitate of calcium oxalate with N/10 potassium permanganate. Acid-insoluble matter, not more than 0.2%. Limit of magnesium and alkalis, weighed as ignited sulphate, not more than 1%. **Calcium Carbonicum Præcipitatum, P. Helv. V**, contains from 98.75 to 100.5% of CaCO_3 . The bulk density of **Calcium Carbonicum Præcipitatum ad usum externum, P. Helv. V**, is controlled by the following test:—Introduce without shaking 25 g. into a 100 ml. graduated cylinder and allow the cylinder to fall lightly on the palm of the hand ten times; the powder should measure not less than 65 ml.

Tabellæ Calcii Carbonatis (N.F. VII). Contain 92.5 to 107.5% of the labelled amount of precipitated calcium carbonate, including all tolerances. Assayed by dissolving a weighed quantity of powdered tablets in dilute hydrochloric acid, filtering and adding ammonium oxalate solution to an aliquot of the filtrate made alkaline with ammonia solution, filtering and washing the residue. The precipitate and filter are transferred to a flask, dilute sulphuric acid and water added and the mixture heated to 70° and titrated with N/10 potassium permanganate.

Creta (B.P.). Dried at 100° it contains not less than 97% of the pure substance and at that temperature loses not more than 1%. It is assayed with excess of N/1 hydrochloric acid and titration with N/1 sodium hydroxide, using methyl orange as indicator. **Creta Præparata, U.S.P. XII**, contains 97%, after drying at 200°. Residue, insoluble in diluted hydrochloric acid and ignited, not more than 2%.

Prepared chalk and the precipitated variety may be readily distinguished by examination under ultra-violet light from a Hanovia lamp with Wood's screen in the dark room. Prepared chalk gives a peach or flesh-coloured fluorescence. Precipitated calcium carbonate shows a smoky, greyish-violet fluorescence. The test can be used for the identification of the chalk used in samples of Maclean's powder.—W. E. Naylor and A. Surfleet, *Pharm. J.*, ii/1936, 261.

Calcium Carbide. CaC_2 . Grey masses, becoming white when moist. Evolves acetylene when brought into contact with water. May be used as a test for, and in the preparation of, absolute alcohol. Requires special storage.

Calcium Cyanamide (Prop. Name NITROLIM) is formed when nitrogen is passed over calcium carbide heated to 1000°: $\text{CaC}_2 + \text{N}_2 = \text{CaCN}_2 + \text{C}$. The nitrogen of same interacts with water under pressure, thus:—

$$\text{CaCN}_2 + 3\text{H}_2\text{O} = \text{CaCO}_3 + 2\text{NH}_3$$

The nitrogen must first be freed from oxygen. This is effected by fractional distillation of liquid air. The above method of fixing atmospheric nitrogen is

the Frank-Caro process. Calcium cyanamide is a black powder, containing 15 to 20% nitrogen and about 20% free lime. As a fertiliser it is valuable on acid or "sour" soils and is usually treated with oil to render it granular and to reduce its dusty and corrosive nature.

Calcii Gluconas (*B.P. Add. I*). $C_{12}H_{22}O_{14}Ca, H_2O = 448.3$. Determined on its calcium content by precipitation as oxalate and ignition with sulphuric acid, it contains from 99% to the equivalent of 104% of the pure substance; 5 ml. of the filtrate obtained from 0.5 g. boiled for 2 minutes with 10 ml. of water and 2 ml. of hydrochloric acid, cooled and precipitated with sodium carbonate, gives no reaction for dextrose or sucrose as indicated by no reduction of potassio-cupric tartrate solution, on boiling for 1 minute. *Calcii Gluconas, U.S.P. XII*, dried over sulphuric acid and determined by precipitation with ammonium oxalate and titration with N/10 potassium permanganate, contains 8.8 to 9.3% of Ca corresponding to 99% of the monohydrate.

A tentative method for the determination of calcium gluconate in preparations, the aqueous solutions of which are neutral and do not contain salts of other optically active hydroxy acids, is described in *Methods of Analysis (A.O.A.C., 1940, 617)*.

Calcii Hydroxidum (*B.P. Add. I*). $Ca(OH)_2 = 74.1$. Assayed by digestion with sucrose solution, filtration, and titration of an aliquot part with N/1 hydrochloric acid to phenolphthalein, when it should contain not less than 90% of $Ca(OH)_2$. Aluminium, iron and matter insoluble in hydrochloric acid, not more than 1.0%, the test to be performed on one-half the quantity of substance. The *U.S.P. XII* substance, by precipitation with excess ammonium oxalate in ammoniacal solution and titration of the acidified precipitate of calcium oxalate with potassium permanganate, contains not less than 95% of $Ca(OH)_2$; hydrochloric acid insoluble matter, not more than 0.5%; limit of magnesium, weighed as ignited sulphate, not more than 4.8%.

Liquor Calcii Hydroxidi (*B.P.*). Contains at 15.5° not less than 0.15% *w/v* of $Ca(OH)_2$. Determined by titration with N/10 sulphuric acid using phenolphthalein indicator. The *U.S.P. XII* preparation contains at 25° not less than 0.14% of $Ca(OH)_2$.

Calx Sodica (*U.S.P. XII*). Contains calcium hydroxide and sodium or potassium hydroxide or both. "Low-moisture Soda Lime" contains less than 9% of moisture; "High-moisture Soda Lime" contains 9 to 19%. It may contain an indicator which changes colour when the capacity of the substance for carbon dioxide is exhausted. Tested for loss on drying, moisture absorption and carbon dioxide adsorption capacity.

Calcii Oxidum (*B.P.C.*). $CaO = 56.08$. Loss on ignition not more than 10%. The ignited substance should contain not less than 95% of CaO when determined by the *B.P.* method for *Calcii Hydroxidum*. *Calx, N.F. VII*, loses not more than 10% on ignition to constant weight with a blast lamp, and then contains not less than 95% of CaO. It is assayed by precipitation with excess N/10 oxalic acid in ammoniacal solution and back titration of the acidified filtrate against N/10 potassium permanganate.

CAMPHORA

Camphora (*B.P.*). $C_{10}H_{16}O = 152.1$. Both the synthetic and natural substances are official. M.p., 174° to 177° . Residue on volatilisation not more than 0.05%. The natural and synthetic products, as Camphora, are official in the *U.S.P. XII*; α_D at 25° , of the natural substance in alcohol, $+41^{\circ}$ to $+43^{\circ}$; residue on sublimation, not more than 0.05%; m.p., 175° to 179° . Camphre Artificiel, *Fr. Cx.* 1937, is assayed by weight of the oxime formed with hydroxylamine hydrochloride. The camphoroxime does not melt below 114° . Camphre du Japon, *Fr. Cx.* 1937, should be used for internal use; rotation of 10% solution in absolute alcohol, at 20° , in 2 decimetre tube, about $+8.60^{\circ}$. Camphora and Camphora synthetica are official in the *P.G. VI*; the former melts at 175° to 179° and has a specific rotation for a 20% solution in absolute alcohol of $+44.22^{\circ}$, whilst the latter melts at not lower than 170° and has a specific rotation of -2° to $+5^{\circ}$.

Purity and Assay. The following standard for camphor is recommended by the Sub-Committee on General Organic Chemicals of the Committee on General Chemistry:—Not less than 97% of $C_{10}H_{16}O$. α_D of natural camphor, in a 10% w/v solution in alcohol (95%), $+40^{\circ}$ to $+42^{\circ}$. **Assay.**—Dissolve about 0.2 g., accurately weighed, in 25 ml. of aldehyde-free alcohol (95%) in a 300-ml. flask; add slowly, with constant shaking, 75 ml. of solution of dinitrophenylhydrazine. Heat on a water-bath under a reflux condenser for four hours, allow to cool, dilute to 200 ml. with a 2% v/v solution of sulphuric acid and set aside for twenty-four hours. Collect the precipitate in a tared Gooch crucible fitted with a paper mat, wash with successive quantities of 10 ml. of cold water until the washings are neutral to litmus paper, dry at 80° , and weigh. Each g. of the residue is equivalent to 0.458 g. of $C_{10}H_{16}O$. **Solution of dinitrophenylhydrazine:** dissolve 1.5 g. of 2:4-dinitrophenylhydrazine in 20 ml. of sulphuric acid (50% v/v), dilute to 100 ml. with water and filter. This solution must be freshly prepared.—(*British Pharmacopœia Commission Report*, No. 14, September 1939).

An official quantitative method for the determination of camphor (optically active) is described in *Methods of Analysis (A.O.A.C., 1940, 575)*.

The above process is also recommended by the Sub-Committee on the Assay of Galenicals of the Committee on Pharmaceutical Chemistry for the assay of camphor in spirit of camphor.

Determination with 2:4-Dinitrophenylhydrazine. The following modification of the method of Hampshire and Page (*Quart. J. Pharm.*, 1934, 558) is recommended. 2 ml. of Tinct. Opii Camph. is diluted with 13 ml. of alcohol 90%, 85 ml. of reagent (1.25% of 2:4-dinitrophenylhydrazine dissolved in 10 ml. of sulphuric acid and 10 ml. of water and diluted to 100 ml. with water and filtered) is added slowly. The mixture is heated under a reflux condenser for 4 hours, cooled, diluted to 200 ml. with 2% v/v sulphuric acid and left in the dark for 24 hours. The precipitate is collected, the flask and precipitate washed six times with 10 ml. of water and the precipitate dried at 80° for 1 hour and weighed. 1 g. of the 2:4-dinitrophenylhydrazine is equivalent to 0.458 g. of camphor.—M. M. Janot and M. Mouton, *J. Pharm. Chim., Paris*, 1936, 23, 547.

Colorimetric Determination. 1 ml. of spirit of camphor is mixed with 3 ml. of alcohol 95% and 2 drops of 1% alcoholic furfural solution added, followed by 2 ml. of concentrated sulphuric acid. The mixture is heated on a water-bath for 5 minutes, and the violet colour is compared with that obtained by treating similarly known amounts of camphor. Benzaldehyde may be used instead of furfural, giving a red colour.—A. Castiglioni, per *Quart. J. Pharm.*, 1937, 228.

Creta cum Camphora. The camphor precipitated by pouring an alcoholic solution into water may contain approximately its own weight of water.

Camphorated chalk prepared by the *B.P.C.* method may contain therefore only 9% of camphor. It is advisable that a minimum limit be fixed.—C. L. M. Brown, *Quart. J. Pharm.*, 1937, 402.

Camphoræ Monobromidum (*B.P.C.*) melts between 74° and 76°. Ash, not more than 0.05%.

Official methods for the determination of camphor monobromide in tablets are described in *Methods of Analysis* (*A.O.A.C.*, 1940, 575).

Leptazolium (*B.P. Add. III*). $C_6H_{10}N_4 = 138.1$. M.p., 57° to 60°. Ash, not more than 0.1%.

Microidentification of leptazol in mixed aqueous solutions containing alkaloids and other substances is described.—V. E. Stewart, *Industr. Engng Chem. (anal. Edn.)*, 1939, 345.

Nikethamidum (*B.P. Add. III*). $C_{10}H_{14}ON_2 = 178.1$. Assayed by digestion with 50% *w/v* nitrogen-free sulphuric acid, distillation of the liberated diethylamine into N/10 hydrochloric acid and back titration to methyl red indicator, a blank distillation being performed; it contains not less than 98% of the pure substance. Sp. gr., 1.064 to 1.067. n_{D20° , 1.525 to 1.526.

Oleum Camphoræ Rectificatum (*B.P.C.*). The f.p. of a mixture of 1.5 g. of the oil, 1.5 g. of eucalyptol and 2.1 g. of *o*-cresol is not below 40°, equivalent to not less than 35% of cineole. Sp. gr. 0.875 to 0.900. $\alpha_D + 9^\circ$ to $+24^\circ$. n_{D40° , 1.465 to 1.470.

The two commercial varieties are white camphor oil and brown camphor oil. The former is the early fraction of the natural oil, obtained as a by-product in the manufacture of camphor; the sp. gr. is lower than 0.998. Brown camphor oil is the higher boiling fraction, the sp. gr. varies from 1.000 to 1.040; oil of the higher figure contains a large proportion of safrole, while oil with a gravity in the neighbourhood of 1.000 is the by-product after removal of the safrole by refrigeration. Camphor oil containing a large proportion of safrole and with a specific gravity of about 1.065 is sold as artificial sassafras oil.

CANNABIS

Cannabis (*B.P.C.*). The drug may now be obtained from plants grown in India, Germany, America and South Africa and contains not more than 10% of fruits, large foliage leaves, and stems over 3 mm. in diameter, and not more than 2% of other foreign organic matter. Acid-insoluble ash limit, 5%. Matter soluble in alcohol after drying at 100°, not less than 10%.

Separation of constituents given in the Supplement to the 4th Report of the L. of N. *Alcohol*.—Shake for one hour 5 g. of sample and 100 ml. of petroleum ether. Filter. Place 80 ml. of filtrate in a separator and extract with 50, 30, 30, 20 and 20 ml. portions of N/10 sodium hydroxide. Wash the petroleum ether solution twice with water and add the washings to the alkaline aqueous solution. Filter the petroleum ether solution through cotton into a tared flask, remove the solvent, place in a desiccator for twelve hours and weigh. This gives the alcoholic extract from 4 g. of sample. *Phenol I*.—Extract the alkaline aqueous solution with 100, 75 and 75 ml. portions of ether. To the combined ethereal solutions add 5 ml. of N sulphuric acid, shake, separate and then wash twice with water. Filter into a tared beaker and proceed as for the alcohol. The residue obtained is the amount of phenol I in 4 g. of sample. *Phenol II*.—To the alkaline aqueous solution remaining after extraction with ether add 20 ml. of N sulphuric acid and extract with 100 ml. of ether. Wash

the ether twice with water, filter into a tared flask and proceed as for the alcohol. The residue obtained is the amount of phenol II in 4 g. of sample. *Phenol III*.—Add 25 ml. of petroleum ether to the phenol residues obtained as above and let stand overnight. Decant the solution, wash the undissolved residue twice with petroleum ether, dry in a current of air and place in a desiccator for 12 hours. The residue, which is mainly obtained from the phenol II fraction, is the phenol III obtained from 4 g. of sample. The alcoholic and phenol II fractions are responsible for the biological activity with the alcoholic fraction which is about 55.5% of the total petroleum ether extract. The alcoholic fraction gives an intense alkaline Beam reaction which therefore indicates the presence of the principal constituent of the resin of cannabis which is responsible for its addictive properties. The phenol II fraction gives the acid Beam reaction, but not always the alkaline. Phenol I does not show constant physiological activity, but it is responsible for the optical activity of the resin of cannabis.—F. de Myttenaere, per *J. Amer. pharm. Ass.*, 1939, 355.

Test for Recognition of Hashish : Beam's Test. The suspected material is extracted with petroleum ether of low boiling-point, and the extract filtered and evaporated to dryness. Both extraction and evaporation should be carried out in the cold. In the presence of a considerable quantity of hashish a marked amount of tar-like residue is obtained, but it is sufficient for the reaction if only a faint yellow stain is left. To the residue a weak alcoholic solution of potash or soda (about N/10) is added and the liquid allowed to evaporate at room temperature. In presence of hashish a rich purple or reddish-purple colour develops, which, on dilution with water, takes on a more bluish cast. Hashish is frequently sold dissolved in fat or oil and for such alcohol is best. Extract of Indian hemp and Ceylon samples did not respond. Samples of ganja, charas and majun from India and a plant grown in Egypt responded perfectly. The ordinary hashish sold in Egypt is largely of Greek origin, and of a large number of samples tested since 1909 not one has failed to respond. Soil, climate, cultivation, and curing influence the chemical composition. The following is suggested as a useful presumptive test to which hashish or other cannabis preparations from India, Egypt, Greece, Sudan, and Uganda all respond. The petroleum ether extract is made as usual, and the evaporation of the solvent is carried out in a short test-tube. To the residue is added a few millilitres of reagent prepared by saturating absolute alcohol with dry hydrogen chloride gas. In the presence of cannabis extract the liquid acquires a bright cherry-red colour which disappears on dilution with alcohol or water. Trials were made with a number of plant extracts and over 200 alkaloids, glycosides, etc., but in no case was a similar reaction obtained. Certain volatile oils—e.g., origanum and santal—give a similar reaction, but the colour is far less intense for similar amounts of material.—W. Beam, Wellcome Res. Lab., Khartoum, per *Chem. & Drugg.*, 1916, 12.

The following modification, depending on the phenolic nature of the substance responsible for the coloration, eliminates a number of vegetable substances which give a similar or an interfering colour. The sample is extracted with light petroleum (b.p. 40° to 60°) and the extract filtered into a separating funnel and extracted once or twice with dilute sodium hydroxide solution. The mixed alkaline extracts are acidified and shaken again with successive small quantities of light petroleum. The light petroleum extracts are mixed, the solvent evaporated in a porcelain dish and the residue treated with a saturated solution of hydrogen chloride in dehydrated alcohol. Myrrh and guaiacum are among the very few substances that still interfere.—L. C. Nickolls, *Analyst*, 1936, 604.

Identification of cannabis by Beam's test is not reliable because of the liability to oxidation of the cannabinol during the evaporation of the solution in light petroleum. This is overcome by adding 3 to 4 drops of alcoholic potash, 5%, to 1 ml. of the light petroleum solution of resin, shaking for a minute and adding 1 ml. of amyl alcohol. The final solution varies in colour from pinkish violet to violet-red, according to the amount of active principle present. Samples of the drug and its preparation up to three years old gave strong reaction to the above test, thus contributing to the opinion that cannabinol is not destroyed.—F. de Myttenaere, per *Quart. J. Pharm.*, 1939, 114.

League of Nations Commission on cannabis concluded that the Beam Test and its modification are at present the only tests indicating the biologically active principles and only these principles of cannabis. The great instability of cannabis and its preparation has not been proved. Light petroleum is still

the best solvent for these principles.—F. de Myttenaere, *J. Pharm. Belg.*, 1939, 21, 571, 597 and 615.

Cannabis may be simply and reliably detected by the following tests: A small portion of the drug is placed in a wide-mouthed container with a small excess of a solution containing benzene (nine parts) and sodium hydroxide (2%) in alcohol (one part) and immediately shaken for 5 seconds. The liquid is then immediately decanted into an evaporating dish. The presence of a cannabinol is indicated by a colour change from slightly yellow to pink within one or two minutes, becoming deeper red after standing a short time. Further, spontaneous evaporation of the reddish liquid leaves a pinkish to violet residue which dissolves to an orange-red solution in strong ammonia and to a violet to almost bluish violet solution in acetone.—A. Viehover, *J. Amer. pharm. Ass.* 1937, 589.

Chemistry of the Hemp Drugs.—A. R. Todd, *Nature, Lond.*, ii/1940, 829.

Cannabinol is *l*-hydroxy-3-*n*-amyl-6 : 6 : 9-trimethyl-6-dibenzopyran. Synthesis described.—R. Adams *et al.*, *J. Amer. chem. Soc.*, 1940, 2204.

Cannabidiol has been obtained in crystalline form as long, white rods; m.p., 66°–67°; α_D at 27°, –125°.—R. Adams *et al.*, *J. Amer. chem. Soc.*, 1940, 294.

It would seem that as regards the constituents, Egyptian hemp resin, containing cannabinol and cannabidiol, occupies an intermediate position between American resin, in which cannabinol seems to be absent, and Indian resin from which cannabidiol has not yet been isolated. Cannabidiol gives a positive Beam test, but is physiologically inactive, thus supporting the view that this test is not specific for the active principle of hashish. A new constituent of Indian hemp has also been isolated and named cannabool. It gives a negative Beam test, but a positive indophenol reaction. It seems probable that it is a partially hydrogenated cannabinol, isomeric with cannabidiol.—A. Jacob and A. R. Todd, *Nature, Lond.*, i/1940, 350.

Determination of Physiological Activity of Hemp Resin. The activity of charas, ganja and bhang (for descriptions of these terms see Vol. I, p. 353) is associated with their resins, but the resin content is not a measure of potency. There is a definite relationship between the specific rotation of the drug and its physiological activity, and a polarimetric method has been devised for its assay. The specific rotation of the resin of good and fresh charas is about –105° whilst that of older and inferior material is as low as –64°; that of ganja of good quality is about –90°, and of inferior quality about –60° or less.—M. N. Ghose and S. W. Bhattacharjee, *Analyst*, 1935, 313.

Extractum Cannabis. This material is sometimes submitted to a biological test by administering doses of a soft extract, equal to 0.25 g., to one or two cats. The extract is given in gelatin capsules which are placed at the back of the mouth and so readily swallowed. Active extracts produce some intoxication and inco-ordination of movement; most striking is the inclination to stay for several seconds in an awkward posture. The effect may last for 3 or 4 hours.

CANTHARIS

Cantharis (B.P.C.). Foreign organic matter, not more than 2%. Moisture, not more than 10%. Cantharis, in powder, is adjusted, by the addition of powder of higher or lower cantharidin content, to contain not less than 0.6% of cantharidin. It is assayed by maceration with chloroform acidified with hydrochloric acid, filtration and evaporation of an aliquot part; after removal of the fat with light petroleum the insoluble residue is treated with N/1 sodium hydroxide and warmed with 5% potassium permanganate solution; the mixture is made strongly acid with 40% sulphuric acid, chloroform and ferrous sulphate are added, and the cantharidin extracted with chloroform; the residue, after evaporation of the chloroform, is finally weighed after drying at 65°. Cantharis, *N.F. VII*, yields not more than 10% of moisture and not less

than 0.6% of cantharidin, by extraction with a mixture of benzene, petroleum ether and hydrochloric acid at 40°; after evaporation of the solvent from an aliquot part, followed by evaporation with chloroform, the residue is washed with a mixture of absolute alcohol and petroleum ether saturated with cantharidin, dissolved in chloroform, evaporated, and dried at 60° for 30 minutes. Cantharides, *P.G. VI*, should yield not less than 0.7% of cantharidin determined by maceration for 24 hours with chloroform and hydrochloric acid (20 : 1), addition of ether and filtration. From an aliquot part most of the solvent is evaporated and the remainder spontaneously, the residue treated with 10 ml. of petroleumbenzene and absolute alcohol (19 : 1), shaken occasionally for 12 hours, the crystalline residue treated with 5 ml. of benzene-alcohol mixture, dissolved in chloroform, evaporated, dried and weighed. Further treatment is necessary if the cantharidin is not crystalline. Cantharis, *P. Helv. V*, contains not less than 0.7% of cantharidin, and the powder when prescribed except for plasters is adjusted with lactose to contain 0.6%. Assayed by refluxing 10 g. with 100 g. of benzene and 1 g. of hydrochloric acid for 30 minutes; 81 g. of filtrate (= 8 g. drug) is evaporated to 5 g. and dried on a water-bath at 60° in a current of dried air. The residue is treated with 10 ml. of petroleum ether absolute alcohol mixture (19 : 1), the crystals collected, dissolved in chloroform, evaporated spontaneously and dried over sulphuric acid.

The following modified process of Self and Greenish is recommended for the assay of cantharidin in cantharides:—Triturate 10 g. in moderately coarse powder with 1 ml. of HCl and exhaust completely in a continuous extraction apparatus with 100 ml. of CHCl_3 . Distil or evaporate off the CHCl_3 and remove the last traces by a current of air. Boil the residue under a reflux condenser with 70 ml. of H_2O and filter immediately through a small moistened filter paper in a warmed separator. Return the fat and filter to the flask and boil for 5 minutes with 50 ml. H_2O ; repeat twice with 50 ml. and 40 ml. of water respectively. Cool the mixed aqueous extract, add 2 ml. of HCl and extract with 30, 25 and 20 ml. of CHCl_3 , wash the CHCl_3 with 10 ml. of H_2O , remove the CHCl_3 and dry the residue in a current of air at 60°. Wash the residue with 3 lots of 5 ml. each of a mixture of 3 parts of light petroleum and 1 part of absolute alcohol previously saturated with cantharidin, dry the residue of cantharidin at 60° and weigh.—G. A. Guthrie and H. Brindle, *Quart. J. Pharm.*, 1942, 61.

Cantharidinum (B.P.). $\text{C}_{10}\text{H}_{12}\text{O}_4 = 196.1$. M.p., 216° to 218°. Ash, not more than 0.1%. Cantharidin in preparations containing it may be detected by the following process:—To 20 ml. of the liquid add sufficient H_2SO_4 to make it slightly acid and extract 3 or 4 times with chloroform. Evaporate the CHCl_3 to about 2 to 3 ml., add 0.2 ml. of a vegetable oil such as sesame or cottonseed, evaporate and dry at 100° to remove the CHCl_3 . Apply to the skin on a very small pad of lint (the lint should be saturated with the oil) and leave in contact for about 12 hours. If cantharidin is present, a blister will form or the skin be much reddened. Taking 20 ml. of a liquid, the test will show 5 to 10 parts of cantharidin per million. If a larger volume is taken the test may, of course, be made still more delicate.

CAPSICUM

Capsicum (B.P.). Contains not more than 3% of calices and pedicels, not more than 1% of stalks and foreign organic matter, and yields not more than 7% of ash. Capsicum, *N.F. VII*, contains not more than 3% of stems and calyces and not more than 1% of other foreign organic matter; non-volatile ether-soluble extractive, not less than 12%; acid-insoluble ash, not more than 1.25%. In the *N.F. VII* the botanical sources of Capsicum are given as *Capsicum frutescens* (African Chillies), *Capsicum annuum* var. *conoides* (Tabasco Pepper), *Capsicum annuum* var. *longum* (Louisiana Long Pepper) and a hybrid between the Honka variety of Japanese Capsicum and Old Louisiana Sport Capsicum (Louisiana Sport Pepper).

Pungency Test. The following test for pungency of capsicum is recommended by the Sub-Committee on Crude Drugs of the Committee on Pharmacy and Pharmacognosy:—Macerate 1 g., accurately weighed, in moderately coarse powder, with 50 ml. of alcohol (95%) shaking continuously for six hours or frequently during twenty-four hours, and filter. Dilute 6 ml. of the filtrate to 100 ml. with water. Dilute 1 ml. of this solution to 50 ml. with a 5% solution of sucrose in water; 3 ml. of this solution, swallowed all at once produces a distinct sense of pungency in the throat of at least two out of three individuals.—(*British Pharmacopæia Commission Report*, No. 13, September 1939.)

The Food and Drug Administration of the U.S. Dept. of Agriculture defined cayenne pepper, cayenne, as the dried, ripe fruit of *Capsicum frutescens* L., *C. baccatum* L., or some other small-fruited species of *Capsicum*. Required to contain not less than 15% of non-volatile ether extract, not more than 1.5% of starch, not more than 28% of crude fibre, not more than 8% of total ash, nor more than 1.25% of ash insoluble in hydrochloric acid.—*S.R.A., F.D., No. 2, Rev. 5, Nov. 1936.*

Extraction of Capsaicin and its Colorimetric Determination. 100 g. of oleoresin (*U.S.P. X*, extracted with ether) is mixed thoroughly with twice its volume of liquid paraffin and shaken out with three successive 200 ml. portions of alcohol (57%). The mixed alcoholic extracts are shaken with 100 ml. of liquid paraffin, the alcoholic layer is separated and the alcohol distilled off. The cooled aqueous residue is extracted with ether, emulsification being prevented by the addition of sodium chloride. The ether is removed by distillation, and the oily residue is boiled for 10 minutes, with occasional stirring, with 4 g. of lithium hydroxide (carbonate-free) and 200 ml. of water. After standing overnight carbon dioxide is passed intermittently through the mixture for two hours, water being added if it becomes too thick, and it is again allowed to stand overnight. The precipitate is collected, washed and dried at a low temperature. The precipitate and filter are boiled under a reflux condenser with 500 ml. of light petroleum for 15 to 20 minutes, the hot light petroleum decanted on to a filter and the clear filtrate set aside below 0° to crystallise. The crystals are rapidly filtered and transferred to a securely stoppered vial. The first precipitate and the filters should be re-extracted with light petroleum. At least 5 g. of capsaicin is obtained from 100 g. of oleoresin of Mombasa capsicums. It has a m.p. of 64°, and its acidity is detectable in a dilution of 1:10,000,000.

Determination. The determination depends on the production of an intense blue colour with vanadium oxychloride. A 2% w/v extract of capsicum (dried overnight in a desiccator) prepared by maceration for 30 to 60 minutes in dry acetone, or a 0.2% solution of the oleoresin in dry acetone is prepared, and also a standard 0.02% solution of capsaicin coloured with an acetone extract of capsaicin-free paprika to match the test solution. Standard tubes are prepared with from 1.5 ml. upwards of capsaicin solution diluted with coloured acetone to 5 ml. To each tube is added one drop of a 1% w/v solution of vanadium oxychloride in carbon tetrachloride for each thousandth part per cent. of capsaicin present. The reagent is then added drop by drop to the unknown until no deepening in colour is observed; excess must be avoided since the colour changes to green. The colour slowly fades after the reaction.—*L. F. Tice, Amer. J. Pharm. 1933 320.*

A study of the oxytrichloride colorimetric method for the determination of capsaicin in capsicum led to the following conclusions: (1) The maceration period of 30 to 60 minutes is sufficient to extract the capsaicin from capsicum using acetone as the solvent. (2) The duration of the reliable colour in the standard is not long enough to ensure satisfactory matching with the unknowns. (3) A permanent non-fading colour for the standard may be achieved by using colorimetric solutions of cupric sulphate and ferric chloride. (4) More concordant results are obtained by using a weaker solution of the vanadium oxytrichloride (0.5% prepared volumetrically), and measuring the reagent from a graduated pipette on a burette instead of using the dropper method. (5) A number of substances containing phenolic compounds gave colour reactions with the reagent. In some instances the colours produced approximated the standard so closely that a colour test using vanadium oxytrichloride would not serve as a reliable qualitative or quantitative test for capsaicin.—A. Hayden and C. B. Jordon, *J. Amer. pharm. Ass.*, 1941, 107.

Oleoresin of Capsicum. The so-called oleoresins of capsicum vary in appearance, solubility and degree of pungency according to the solvent used for extraction.

Oleoresin of capsicum, *B.P.C.*, is prepared by extracting an ether oleoresin with alcohol (90%) and is soluble in ether, alcohol (90%), acetone, benzene, chloroform, petroleum spirit, fixed oils and turpentine. It has a greater pungency value than any of the other oleoresins of capsicum, the pungency being about three or four times that of oleoresin of capsicum, *B.P.C.*, '23, which was extracted with alcohol (60%).—H. Berry, *Quart. J. Pharm.*, 1935, 479.

CARBO

Carbo. (B.P.C.). Ash, not more than 7%. Moisture, not more than 15%. *Carbo Activatus, U.S.P. XII*, loses not more than 15% at 120°; ash, not more than 4%; acid-soluble substances, not more than 3.5%; tests for adsorptive power using strychnine sulphate solution, methylthionine chloride and sodium sulphide are included; a test for heavy metals is included. *Carbo Animalis Purificatus, N.F. VII*, loses not more than 12% at 100° for 2 hours; hydrochloric acid-soluble impurities, not more than 6%; tests for adsorptive power with methylene blue and with N/10 iodine are included.

A test for activity of medicinal and other charcoals by exposure to water and other vapours. Active charcoal will absorb 50 to 100%, or even more, of moisture. The water figure is slightly higher than that for alcohol or turpentine. *Pulv. Carbo. Lig.* as ordinarily dispensed for medicinal purposes is inactive. The author suggests improvements in the manufacture, and the adoption in the *B.P.* of tests for activity.—H. Brindle, *Pharm. J.*, ii/1928, 84.

Astonishing variety in the potency of different brands. A comparison of the adsorptive powers of thirty samples (using methylene blue as the substance to be adsorbed) showed coefficients varying from 85 to 0.5.—N. Mutch, *Brit. med. J.*, i/1934, 320.

Tests for Purity. The following tests for purity for medicinal charcoal are recommended by the Sub-Committee on Ointments and Miscellaneous Galenicals of the Committee on Pharmacy and Pharmacognosy:—It adsorbs from solution not less than 30% of its weight of phenazone and adsorbs completely from solution 10% of its weight of strychnine hydrochloride, both calculated with reference to its activated charcoal previously heated to 110°. A black, odourless and tasteless powder, free from gritty particles. Unchanged when heated in the absence of air. When burnt in air or oxygen, forms carbon dioxide and carbon monoxide. When heated with sulphuric acid, evolves carbon dioxide and sulphur dioxide. The Tests for Purity should include the following:—When heated, gives off no foreign odour (absence of adsorbed vapours). Boil 1 g. with 50 ml. of water for five minutes under a reflux condenser, cool, filter, and reject the first 15 ml. of the filtrate; 25 ml. of the filtrate

requires for neutralisation not more than the 0.2 ml. of either N/10 sulphuric acid or N/10 sodium hydroxide, solution of bromothymol blue being used as indicator (limit of alkalinity or of acidity). Boil 0.25 g. with 10 ml. of solution of sodium hydroxide and filter; the filtrate is colourless. Boil 0.5 g. with 25 ml. of dilute hydrochloric acid; the vapour evolved does not blacken lead paper (limit of sulphide). Boil 1.5 g. for five minutes with 100 ml. of water under a reflux condenser; cool, filter, and reject the first 15 ml. of the filtrate:—10 ml. of the filtrate complies with the limit test for chlorides; 30 ml. of the filtrate complies with the limit test for sulphates. Add 5 g. to a solution of 2 g. of tartaric acid in 50 ml. of water contained in a distillation flask. Attach the flask to an efficient condenser with the outlet dipping below the surface of 10 ml. of N/1 sodium hydroxide, and distil about 25 ml. To the liquid in the receiver add 2 ml. of solution of ferrous sulphate and then acidify with hydrochloric acid: no blue colour or precipitate is produced (absence of cyanogen compounds). Boil 1 g. with a mixture of 20 ml. of dilute hydrochloric acid and 5 ml. of solution of bromine for five minutes; filter, and wash the residue with 50 ml. of boiling water; evaporate the filtrate and washings to dryness and treat the residue with a mixture of 1 ml. of N/1 hydrochloric acid, 20 ml. of water and 5 ml. of solution of sulphurous acid; boil until all the sulphur dioxide is expelled and dilute to 50 ml. with water; the solution complies with the following tests:—10 ml. shows no change of colour within ten seconds when 5 ml. of solution of hydrogen sulphide is added. 10 ml. boiled with 0.3 ml. of nitric acid, again boiled with excess of dilute solution of ammonia, and filtered, if necessary, gives only a slight discoloration with 5 ml. of solution of hydrogen sulphide within ten seconds, and no turbidity within two minutes (limit of heavy metals). Loses, when heated at 110°, not more than 10% of its weight. Leaves, on ignition, not more than 4% of residue. *Assay*.—For adsorptive power. Add about 0.3 g. accurately weighed, to 50 ml. of a 0.4% solution of phenazone in a stoppered flask, and shake at frequent intervals for twenty minutes. Filter through a dry filter paper and reject the first 15 ml. of the filtrate. To 25 ml. of the filtrate in a stoppered flask add 2 g. of sodium acetate and 30 ml. of N/10 iodine and shake occasionally for twenty minutes. Add 10 ml. of chloroform, shake until the precipitate is dissolved and titrate the excess of iodine with N/10 sodium thiosulphate. Repeat the process, omitting the activated charcoal. The difference between the two titrations corresponds to the amount of iodine required for the phenazone adsorbed by the activated charcoal. Each ml. of N/10 iodine is equivalent to 0.009405 g. of phenazone. For complete adsorptive power. Shake the equivalent of 1 g. of the activated charcoal dried at 110° with 50 ml. of a 0.2% solution of strychnine hydrochloride in water for five minutes, filter through a dry filter paper, and reject the first 15 ml. of the filtrate; 10 ml. of the filtrate gives no turbidity or precipitate on the addition of 0.5 ml. of dilute hydrochloric acid and 0.5 ml. of solution of potassium-mercuric iodide.—(*British Pharmacopæia Commission Report*, No. 13, September 1939).

Carboni Dioxidum (*B.P. Add. I*). $\text{CO}_2 = 44.00$. By measurement of the volume adsorbed when passed into 50% *w/v* potassium hydroxide solution at N.T.P. it contains not less than 99% *v/v* of CO_2 . 500 ml. (at N.T.P.) is passed through 50 ml. of sodium bicarbonate solution and then through 80 ml. of water with 4 drops of methyl orange; a further 500 ml. is passed directly through one-half of this solution, when the colour should not differ from that of the other half (limit of acid and sulphur dioxide). In the limit test for phosphine, hydrogen sulphide and organic reducing substances, silver ammonio-nitrate solution is replaced by a mixture of silver nitrate and dilute ammonia solution and water. The *U.S.P. XII* fixes the same standard and assays similarly. 1000 ml. passed through 50 ml. of recently boiled and cooled distilled water, under specified conditions, renders it not more acid to methyl orange than 1 ml. of N/100 hydrochloric acid. Carbon monoxide is excluded by shaking 1000 ml. with diluted blood and adding a mixture of pyrogallol and tannic acid.

The grey colour obtained is matched against that produced in a blank test using 1000 ml. of carbon monoxide-free carbon dioxide.

Carbon Monoxide in Industry.

This gas occurs when any organic matter is burnt in a restricted amount of air and hence is a component of town gas (6 to 9%) and the exhaust gases of motor engines. It is also one of the components in producer or water gas (25 to 50%) and carburetted water gas (30%). Considerable amounts of carbon monoxide are liberated when explosives are detonated and in war-time there is grave risk of poisoning in camouflages, where bombs have exploded underground, and which are filled with carbon monoxide under pressure. It is colourless, odourless and non-irritant and herein lies its chief danger, since often the first warning of its presence may be inability to move. Its poisonous action is due to its power of combining with the hæmoglobin of the blood, forming a compound which is not decomposed by oxygen. Its affinity for hæmoglobin is about 250 times that of oxygen, and hence in the presence of both gases the partition of hæmoglobin is strongly in favour of combination with carbon monoxide. Thus, as the absorption of carbon monoxide by the blood proceeds the supply of oxygen to the blood progressively decreases.

Table of Permissible Concentrations of CO in Air.

| | |
|---|----------------|
| Allowable for exposure of several hours | 0.01% |
| Allowable for exposure of one hour without appreciable effect | 0.04 to 0.05% |
| Causing just appreciable effect after one hour | 0.06 to 0.07% |
| Causing unpleasant but not dangerous symptoms after one hour | 0.1 to 0.12% |
| Dangerous for exposure of one hour | 0.15 to 0.2% |
| Fatal in exposure of less than one hour | 0.4% and over. |

—V. Henderson and F. W. Haggard, per *Lancet*, i/1933, 154.

Treatment. Remove patient into fresh air at once, keep him lying down and warm, and apply artificial respiration, keeping it up steadily for hours if necessary. Give inhalations of oxygen with 5 to 7% carbon dioxide. Methylene blue, 50 ml. of 1% solution intravenously, said to be of value. Stimulants such as hot black coffee. Nikethamide, 5 to 15 ml. of 25% solution intravenously, said to be useful. Venesection not to be recommended as it withdraws healthy as well as poisoned blood and weakens the patient, but blood transfusion has sometimes saved life. Saline infusion. The patient should be kept quiet for several days and carefully watched in case fresh symptoms appear.

Detection and Determination. The most satisfactory method for detecting and determining carbon monoxide in the air is by the use of palladium chloride. A 0.5% solution of palladium chloride in distilled water is filtered bright and mixed with an equal volume of A.R. acetone. Pieces of filter paper are soaked in the mixed reagent, pressed very lightly to expel excess solution, and known volumes of the suspected air drawn through by means of a pump or by a glass bottle aspirator. In order to remove hydrocarbons, especially when exhaust fumes are suspected, the air is

drawn through a tube containing activated carbon, before being passed through the treated filter paper, carbon monoxide not being absorbed by charcoal. A grey to black colour denotes the presence of carbon monoxide, owing to the reduction to palladium, and according to the volume of air filtered and the depth of colour the amount of CO in the air can be determined. It is inadvisable to keep the palladium chloride solution mixed with acetone, and the filter papers must be prepared immediately before use.—(*Toxic Gases in Industry*, No. 7, D.S.I.R.)

Phosgene in Industry.

Phosgene or carbonyl chloride (COCl_2) is usually considered to be one of the most powerful lung irritant war gases, but it is also used as an intermediate in several chemical processes and hence the risk is not restricted to chemical warfare. It may also occur as the result of incomplete combustion of organic solvents, used as fire extinguishers, such as carbon tetrachloride and trichlorethylene. It is commonly stated to smell like musty hay, but many forms of over-ripe vegetation, such as freshly cut sour apples, resemble it closely. It is a colourless gas, boiling at 8.2°C . and is, therefore, non-persistent. One of the most unfortunate aspects of poisoning by this gas is that there may be slight coughing, lachrymation and tightness of the chest at first, but that these soon pass off, leaving the patient with a feeling of well-being, to be followed later by a severe collapse, due to severe oedema of the lungs, though the face may not appear fully cyanosed as with most lung irritants. For prolonged exposure the greatest possible concentration is 1 part in 1,000,000, whilst 1 part in 200,000 for half an hour may prove fatal eventually. It is readily absorbed by activated carbon and decomposed by many chemical agents such as water, alkalis and hexamine.

Detection and Determination. Apart from the odour, phosgene is most readily detected by the development of a yellow to deep orange colour on a paper impregnated with a solution containing 5% each of *p*-dimethylaminobenzaldehyde and diphenylamine in absolute alcohol, and dried. The colour developed may be compared with standard colour charts.—(*Toxic Gases in Industry*, No. 8, D.S.I.R.)

Carbonei Disulphidum (B.P.C.). $\text{CS}_2=76.12$. Sp. gr., 1.268 to 1.272; residue on evaporation at 100° , not more than 0.01%; boiling range, 95% between 46° and 47° .

The B.S.S. No. 662—1936 includes requirements for specific gravity (1.270 to 1.274 at 15° and 1.265 to 1.269 at 20°), boiling range (not less than 95% below 46.4°), residue on evaporation and acidity, and a test for absence of hydrogen sulphide.

Detection in the Atmosphere. Samples of the air under examination are bubbled through a mixture of 10 ml. of absolute alcohol, 2 ml. of solution (a) containing 2 ml. of diethylamine in 100 ml. of benzene, and 2 ml. of solution (b) containing 0.1 g. of copper acetate in 100 ml. of absolute alcohol. After standing, the colour developed is compared with a series of standards of known strengths of carbon disulphide.—(*Toxic Gases in Industry*, No. 6, D.S.I.R.)

CARDAMOMUM

Cardamomum (B.P.). Separated from the fruits when required for use, and contains not more than 3% of foreign organic matter. Ash, not more than 6%. *Cardamomi Semen*, *U.S.P. XII*, should be recently removed from the capsules and yield not more than 4% of acid-insoluble ash.

The *B.P.* limit of impurity should apply to the fruit and not to the seed, which must not be separated from the fruit until required for use and is therefore protected from adulteration.—*Pharm. J.*, ii/1932, 68.

The Food and Drug Administration of the U.S. Dept. of Agriculture defined cardamom as the dried, nearly ripe fruit of *Elettaria cardamomum* Maton. Cardamom seed was defined as the dried seed of cardamom. Required to contain not more than 8% of total ash, nor more than 3% of ash insoluble in hydrochloric acid.—*S.R.A., F.D., No. 2, Rev. 5, Nov. 1936.*

Oleum Cardamomi (B.P.C.). Sp. gr., 0.923 to 0.945; α_D^{20} , +20° to +44°; n_{D20° , 1.461 to 1.467. Ester value, 90 to 156. It should be soluble in 4 volumes of alcohol (70%, sp. gr. 0.8896 to 0.8901). *Oleum Cardamomi, N.F. VII*, has a sp. gr. at 25° of 0.917 to 0.947. Rotation in a 100 mm. tube at 25°, +22° to +44°.

The husks are for practical purposes inert. The green cardamoms yield more oil than the bleached. Imported decorticated seeds yield less oil than those recently removed from the husk. Loss of oil from the husk-protected seed on keeping over eight months is small; decorticated seeds lost 80% of oil on keeping over the same period. The oil requires not less than three hours for saponification.—Clevenger, *J. Ass. off. agric. Chem., Wash.*, 1934, 283.

CARUM

Carum (B.P.). Limits: for foreign organic matter, 2%; for ash, 9%; for acid-insoluble ash, 1.5%. *Carum, U.S.P. XII*, contains not more than 3% of other fruits, seeds or foreign organic matter; acid-insoluble ash, not more than 1.5%. *Fructus Carvi, P.G. VI*, must yield not less than 4% of volatile oil.

The Food and Drug Administration of the U.S. Dept. of Agriculture defined caraway, caraway seed, as the dried fruit of *Carum carvi* L. Required to contain not more than 8% of total ash or more than 1.5% of ash insoluble in hydrochloric acid.—*S.R.A., F.D., No. 2, Rev. 5, Nov. 1936.*

Oleum Cari (B.P.). Assayed by the same method as for *Oleum Anethi, B.P. '32*, it contains from 53 to 63% *w/w* of carvone. Sp. gr., 0.910 to 0.920. α_D^{20} , +70° to +80°. n_{D20° , 1.485 to 1.492. Soluble in one volume of alcohol (90%, sp. gr. 0.8334 to 0.8340) and in 7 volumes of alcohol (80%, sp. gr. 0.8634 to 0.8640). *Oleum Cari, N.F. VII*, should contain not less than 50% *v/v* of carvone; estimated by measurement of the oil remaining after treatment with sodium sulphite. n_{D20° , 1.484 to 1.488.

Determination of carvone in caraway oil.—Bennett and Cocking (*Quart. J. Pharm.*, 1931, 580) give a method of applying the hydroxylamine reaction.

Caraway oil consists mainly of carvone, sp. gr. 0.964, and limonene, sp. gr. 0.846; the sp. gr. of the oil is therefore a good indication of the proportion of carvone.

CARYOPHYLLUM

Caryophyllum (B.P.). Clove should contain not more than 5% of its stalks and not more than 1% of foreign organic matter. Limits: ash, 10%; acid-insoluble ash, 0.75%. Tests for limit of stalks and absence of clove fruits and cereals are included. Caryophyllus, *U.S.P. XII*, should yield not less than 16% *v/w* of volatile oil and not more than 10% of crude fibre; the limits for stem, foreign organic matter and acid-insoluble ash are the same as in the *B.P.* Flores Caryophylli, *P.G. VI*, should yield not less than 16% of volatile oil. Flos Caryophylli, *P. Helv. V*, should yield not less than 16% of essential oil; ash, not more than 7%; acid-insoluble ash, not more than 1%.

The Food and Drug Administration of the U.S. Dept. of Agriculture defined cloves as the dried flower-buds of *Caryophyllus aromaticus* L. Contains not more than 5% of clove stems, not less than 15% of volatile ether extract, not less than 12% of quercitannic acid (calculated from the total oxygen absorbed by the aqueous extract), not more than 10% of crude fibre, not more than 7% of total ash, and not more than 0.5% of ash insoluble in hydrochloric acid.—*S.R.A., F.D., No. 2, Rev. 5, Nov. 1936.*

The export of cloves from Zanzibar is controlled by a Clove Growers' Association. There are standards for three different grades and each bale or package must be up to the standard for the particular grade.—*Perfum. essent. Oil Rec., 1934, 239.*

Oleum Caryophylli (B.P.). By difference from the volume of the oil unabsorbed by potassium hydroxide solution, a eugenol content of not less than 85% and not more than 90% *v/v* should be indicated. Sp. gr., 1.047 to 1.060. n_{D20}° , 1.528 to 1.537. Soluble in 2 volumes of alcohol (70%, sp. gr. 0.8896 to 0.8901). Oleum Caryophylli, *U.S.P. XII*, should contain not less than 82% *v/v* of eugenol, the absorption of the eugenol with potassium hydroxide being effected during 10 minutes at the temperature of the water-bath. The optical rotation should not exceed $-1^{\circ} 30'$ in a 100 mm. tube at 25° ; n_{D20}° , 1.5300 to 1.5350; hot water shaken with the oil is only very faintly acid and on cooling shows no blue or violet colour of phenol with 1 drop of ferric chloride solution. Essence de Girofle, *Fr. Cx. 1937*, by extraction with petroleum ether, transferring to sodium hydroxide, acidifying and extracting with ether, contains not less than 70% of eugenol.

Emulsification of the oil in the assay with caustic potash can be prevented by pretreating the oil with a paste of tartaric acid and then drying over anhydrous sodium sulphate.—P. A. Rowan and J. A. Insinger, *Chem. Weekbl.*, 1939, 36, 642.

Clove oil may contain up to 20% of acetyl-eugenol, which is hydrolysed so easily that it appears as eugenol in the assay for phenols.

Oils having a eugenol content of more than 90% are obtainable but are less fragrant.—C. T. Bennett, *Pharm. J.*, i/1933, 150.

Oil of clove roots. The yield of oil is about 6% from the roots; the eugenol content is high.—*Chem. & Drugg.*, i/1923, 136. Oil distilled from clove stems is also usually high in eugenol.

Eugenol (B.P.C.). $C_{10}H_{12}O_2 = 164.1$. Sp. gr., 1.072 to 1.074; n_{D20}° , 1.541 to 1.542. Eugenol, *U.S.P. XII*, has a sp. gr. at 25° of 1.064 to 1.070 and boils between 250° and 255° ; n_{D20}° , 1.540 to 1.542; optically inactive.

Oleum Myrciæ (B.P.C.). Contains not less than 45% of phenols. Sp. gr., 0.945 to 0.990; α_D , 0° to 4°; n_{D20} , 1.500 to 1.520. Estimated by the B.P. method for eugenol in Oleum Caryophylli. The N.F. VII oil should yield 50 to 65% v/v of phenols, as indicated by the potassium hydroxide absorption; rotation, laevorotatory but not exceeding -3° in a 100 mm. tube at 25°; sp. gr., 0.950 to 0.990 at 25°.

Pimenta (B.P.C.). Yields not more than 6% of ash.

The Food and Drug Administration of the U.S. Dept. of Agriculture defined allspice, pimento, as the dried, nearly ripe fruit of *Pimenta officinalis* Lindl. Required to contain not less than 8% of quercitannic acid (calculated from the total oxygen absorbed by the aqueous extract), not more than 25% of crude fibre, not more than 6% of total ash or more than 0.4% of ash insoluble in hydrochloric acid.—S.R.A., F.D., No. 2, Rev. 5, Nov. 1936.

Oleum Pimentæ (B.P.C.). Eugenol content, determined as for Oleum Caryophylli, not less than 60% v/v; sp. gr., 1.035 to 1.057; α_D , 0° to -5°; n_{D20} , 1.500 to 1.536. Oleum Pimentæ, N.F. VII, should contain 65% v/v of phenols. Sp. gr. at 25°, 1.018 to 1.048; α_D , at 25°, 0° to -4°. The presence of clove oil may be detected by the test for furfuraldehyde, *vide* test under Ol. Menth. Pip.

CASCARA SAGRADA

Cascara Sagrada (B.P.). Foreign organic matter, not more than 2%. Ash, not more than 6%. The U.S.P. XII allows 4% of foreign organic matter, and for medicinal preparations it should be collected one year before use.

The larger proportion of anthraquinone derivatives in cascara are in the combined form. The U.S.P. fluid extract contained 0.4%. Fluid extracts of commerce contained 0.17 and 0.24%. Debittered cascara contained 0.07%.—J. Amer. pharm. Ass., 1926, 847.

The characteristic aperient action is not due to emodin. Emodin is, however, a constituent, but chrysophanic acid or chrysarobin could not be found. Apparently there are no chemical differences between one and three years' old ("matured") bark. Storage was said to exhaust a ferment and to moderate the gripping action which the fresh bark possesses. No relation between the emodin content and the physiological action.—J. D. v. d. Graaf, *Pharm. Weekbl.*, 1932, 753.

The following test for identity of cascara bark is recommended by the Subcommittee on Crude Drugs of the Committee on Pharmacy and Pharmacognosy:—Moisten 0.2 g., in powder, with 1 ml. of alcohol (95%), add 10 ml. of water, and shake for fifteen minutes, boil, cool and filter. Shake the filtrate with 10 ml. of ether; a yellow ethereal solution separates on standing. Shake 3 ml. of the ethereal solution with 3 ml. of dilute solution of ammonia and to the separated aqueous liquid add 20 ml. of water; the mixture retains a characteristic yellowish-red colour (distinction from frangula bark).—(*British Pharmacopæia Commission Report*, No. 13, September 1939.)

A tentative method for the determination of cascara sagrada is described in *Methods of Analysis* (A.O.A.C., 1940, 595).

Substitutes. The bark from Texas, Arizona, Colorado, and New Mexico is sometimes substituted by or mixed with the bark of *Rhamnus Californica*, which is of a greyer tint externally, and the transverse section is less dark and more yellow than *R. Purshiana*. An inferior variety, known as Winter Bark, is cut from the steamed branches and is therefore in the form of chips.—*Chem. & Drugg.*, i/1925, 560.

CHLORALIS HYDRAS

Chloralis Hydras (B.P.). $\text{CCl}_3 \cdot \text{CH}(\text{OH})_2 = 165.4$. Should contain not less than 99% of the pure substance. Assayed by addition of excess of N/1 sodium hydroxide, standing for 2 minutes, and back titration with N/1 sulphuric acid to phenolphthalein. Ash, not more than 0.05%. The *U.S.P. XII* requires a purity of 99.5%.

Assay. The following process for the assay is recommended by the Subcommittee on Synthetic Chemicals:—Dissolve about 4 g., accurately weighed, in 10 ml. of water, and add 30 ml. of N/1 sodium hydroxide. Allow the mixture to stand for two minutes, and titrate with N/1 sulphuric acid, using solution of phenolphthalein as indicator. Titrate the neutralised liquid with N/10 silver nitrate, using solution of potassium chromate as indicator. To the amount of N/1 sulphuric acid used in the first titration, add one-tenth the amount of N/10 silver nitrate used in the second titration, and deduct the figure so obtained from that of the N/1 sodium hydroxide added. Each ml. of N/1 sodium hydroxide represented by the difference is equivalent to 0.1654 g. of $\text{C}_2\text{H}_3\text{O}_2\text{Cl}_3$.—(*British Pharmacopœia Commission Report*, No. 11, May 1939.)

B.P. assay is unsatisfactory due to the hydrolysis of the chloroform produced by the action of the sodium hydroxide. The following method is recommended. Introduce about 0.5 g. of chloral hydrate into a Kjeldahl flask with the aid of 20 ml. of neutral alcohol. Add accurately 25 ml. of 2N alcoholic potash solution and boil gently under reflux for half an hour. Remove the flame, wash the condenser with 15 to 20 ml. of alcohol and boil gently for a further half an hour. After cooling, titrate the mixture with N/2 or N/1 acid to phenolphthalein.—T. L. Bowyer, *Chem. & Drugg.*, ii/1939, 343.

Chloralformamidum (B.P.C. Supp. V). $\text{C}_3\text{H}_4\text{O}_2\text{NCl}_3 = 192.4$. M.p., 116° to 122° . Volatilises without evolving inflammable vapours and leaves not more than 0.01% of residue.

Butylchloralis Hydras (B.P.C.). $\text{C}_4\text{H}_9\text{O}_2\text{Cl}_3 = 193.4$. Ash, not more than 0.05%. Tests for limit of chloride and of chloral hydrate are described.

Chlorbutol (B.P.). $\text{C}_4\text{H}_7\text{OCl}_3 = 177.4$. M.p., not below 78° . M.p. of the anhydrous substance, 96° . Ash, not more than 0.1%. The majority of samples melt slightly below the B.P. minimum. Chlorobutanol, *U.S.P. XII*, should not melt below 76° .

Alcohol Trichlorisobutylicus, *P. Helv. V*, is $\text{C}_4\text{H}_7\text{OCl}_3 \cdot \frac{1}{2}\text{H}_2\text{O}$, and, without previous drying, melts between 79.5° and 81° . It is assayed as follows:—Dissolve about 1 g. in 20 ml. of alcohol and dilute to 100 ml. with water. Transfer 10 ml. to a 200 ml. flask, add 0.5 ml. of 30% sodium hydroxide solution, 10 ml. of alcohol and heat on a water-bath just to boiling. Allow to cool, add 0.5 ml. of concentrated nitric acid, dilute with 50 ml. of water, neutralise with excess of calcium carbonate and titrate the mixture to potassium chromate with N/10 silver nitrate. A blank experiment is also prescribed. Each ml. of N/10 silver nitrate is equivalent to 0.0062147 g. of $\text{C}_4\text{H}_7\text{OCl}_3 \cdot \frac{1}{2}\text{H}_2\text{O}$.

Determination. To avoid the difficulties caused by the presence of organic materials (as in suppositories) the material should be first steam-distilled, the distillate being collected in N/2 potassium hydroxide in propyl alcohol. If the material is alkaline, excess boric acid is added first. The distillate is refluxed for one hour, evaporated in a nickel crucible to dryness, ignited and the chlorine assayed argentimetrically.—G. Vastogh, *Pharm. Zentrall.*, 1937, 78, 498.

Tentative methods for the determination of chlorbutol crystals and of solutions of chlorbutol are described in *Methods of Analysis (A.O.A.C., 1940, 602)*.

CHLORAMINA

Chloramina (B.P.). $\text{C}_7\text{H}_7\text{O}_2\text{NCISNa} \cdot 3\text{H}_2\text{O} = 281.6$. Contains from 98% to the equivalent of 103% of the pure hydrated

compound. Assayed by interaction with potassium iodide in acidified solution for 10 minutes, followed by titration of the liberated iodine with sodium thiosulphate; 1 ml. of N/10 thiosulphate is equivalent to 0.01408 g. of $C_7H_7O_2NCISNa, 3H_2O$. Chloramina-T, *U.S.P. XII*, should contain from 11.5 to 13% of active chlorine, assayed by digestion with potassium iodide in acetic acid solution. 1 ml. of N/10 thiosulphate is equivalent to 0.001773 g. of active chlorine; 0.2 g. complies with a test for carbonisable substances. Chloramine-T, *Fr. Cx. 1937*, contains 24 to 26% of chlorine. Natrium sulfaminochloratum, *P. Helv. V*, contains 24 to 26% of active chlorine.

Chloramine-T has been suggested to replace the more expensive iodine solution in analytical processes.—*J. chem. Soc. Abstr.*, ii/1925, 66.

Carbasus Chloraminæ (B.P.C.). Contains from 4 to 6% of $C_7H_7O_2NCISNa, 3H_2O$.

Dichloramina (B.P.C.). $C_7H_7O_2NCl_2S = 240.0$. Determined by titration of the iodine liberated from potassium iodide in acetic acid solution, it contains not less than 93% of $C_7H_7O_2NCl_2S$; 1 ml. of N/10 thiosulphate is equivalent to 0.006001 g. of $C_7H_7O_2NCl_2S$. Dichloramina-T, *N.F. VII*, should contain not less than 28% and not more than 30% of active chlorine.

CHLOROFORMUM

Chloroformum (B.P.). $CHCl_3 = 119.4$. Sp. gr., 1.485 to 1.490. Usually not more than 15% *v/v* distils below 60°, the remainder distilling between 60° and 62°. Limit of residue on evaporation, 0.004% *w/v*. Tests for acidity, chloride, free chlorine, hydrochloric acid, foreign organic matter, foreign chlorine compounds, decomposition products and aldehyde are included. Chloroformum, *U.S.P. XII*, leaves not more than 0.002% *w/v* of residue, dried at 100°; it contains from 99 to 99.5% of $CHCl_3$. Sp. gr. at 25°, 1.474 to 1.478. Chloroforme Anesthésique, *Fr. Cx. 1937*, should contain 0.5% of alcohol; determined by shaking with sulphuric acid, dilution and distillation, finally titrating with a solution of potassium dichromate. Chloroformum ad narcosin, *P. Helv. V*, is required to be of a higher degree of purity than Chloroformum which is administered in solution; two independent series of control tests are prescribed for these substances.

Determination. Colorimetric determination of small quantities in solution (0.1 to 0.0001%) in animal tissues. A pink colour is obtained on heating a solution with pyridine in presence of sodium hydroxide.—*W. H. Cole, J. biol. Chem.*, 1926, 71, 173.

Chloroform in various mixtures and galenicals may be determined colorimetrically by means of the colour reaction that it gives with β -naphthol in strong potassium hydroxide solution. 10 ml. of 2% *w/v* solution of β -naphthol in 40% cold potassium hydroxide solution is measured in a series of Nessler tubes, measured volumes of a 0.5% standard solution of chloroform in 95% industrial methylated spirit, and sufficient industrial methylated spirit to make the total volume measure 11 ml. are added; the tubes are shaken and allowed to stand

for 5 to 10 minutes, and the colours compared by means of a Dubosq colorimeter. If the colours are compared within a few minutes, good results are obtained.—W. G. Moffitt, *Analyst*, 1933, 2.

Chloroform may be determined in mixtures free from other chlorine compounds (other than chlorides which may be determined and subtracted from the results obtained). Distil the chloroform from a flask containing the mixture and alcohol with a little calcium carbonate into alcoholic potash until about three-quarters of the original volume has been distilled. Allow the alcoholic potash and distillate, after shaking well, to stand in a closed vessel overnight, acidify and determine the chloride by titration with excess silver nitrate, filtration, and back titration with ammonium thiocyanate.

Tentative methods for the determination of chloroform in mixtures are described in *Methods of Analysis (A.O.A.C., 1940, 602)*.

Tests for Phosgene. To 15 ml. of medicinal chloroform (which contains alcohol) in a dry stoppered bottle add 20 mg. each of resorcinol and vanillin. Close the bottle and, when the reagents are dissolved, place it in the dark for 1 hour. Add 5 ml. of 1% aqueous ammonia, shake, and allow to separate. In presence of phosgene or hydrochloric acid a pink or red colour develops in the aqueous layer, reaching a maximum in 30 seconds.—N. L. Allport, *Analyst*, 1931, 706.

Mix with a 1% solution of *p*-dimethylaminobenzaldehyde and diphenylamine in acetone. In the presence of 0.01% of phosgene a yellow colour develops in 15 minutes.—L. Rosenthaler, *Pharm. Acta Helvet.*, 1937, 6.

Aqua Chloroformi (B.P.). A 0.25% solution of chloroform in distilled water. The U.S.P. XII water is a saturated solution and is approximately twice the strength.

Linimentum Chloroformi (U.S.P. XII). Contains at 25°, 27 to 35% *v/v* of CHCl_3 , determined by a standardised fractional distillation method.

Trichloroethylenum (U.S.P. XII). Contains 99 to 99.5% of C_2HCl_3 , the remainder consisting of alcohol. Sp. gr. at 25°, 1.456 to 1.462, B.pt. 86° to 88°, non-volatile residue, dried at 110°, not more than 0.002%. 20 ml. should be equivalent to not more than 0.5 ml. N/100 sodium hydroxide in a test in which phenolphthalein is used as indicator. Tests for free chlorine and chloride ion are included.

Determination. Trichloroethylene is quantitatively hydrolysed by heating with 25% aqueous potassium hydroxide in a sealed Carius tube for 1 hour at 150°. The ratio by weight of potassium hydroxide to trichloroethylene should be 2 or 3 to 1 (i.e., 8 to 12 ml. of the hydroxide solution per g. of trichloroethylene present). Potassium chloride is formed in amount equivalent to the total chlorine present and is determined by the Volhard method.—D. F. Kelly, M. O'Connor and J. Reilly, *Analyst*, 1941, 489.

A British Standard Specification (B.S.S. No. 580—1934) has been issued by the British Standards Institution for trichloroethylene (technical and stabilised). The specification includes requirements regarding description, specific gravity (1.469 to 1.475 at 15.5°), distillation range, residue on evaporation, acidity, free chlorine and sampling, and the appendices describe the methods and apparatus to be used.

Tetrachlorethylene. Determination in mixtures.—*Methods of Analysis (A.O.A.C., 1940, 604)*.

Test. Warm 2 drops with 5 drops of 10% sodium hydroxide solution and 10 drops of pyridine, the pyridine layer assumes a strong red colour.—B. Samdahl, and H. Sansaker, *Norsk. farm. Tidsskt.*, 1938, 46, 31, 46.

Carbonei Tetrachloridum (B.P.). $\text{CCl}_4 = 153.8$. Sp. gr., 1.603 to 1.606; boiling-range, 76.5° to 77.5°. Residue on evaporation on a water-bath, not more than 0.002% *w/v*. The U.S.P. XII substance after evaporating nearly to dryness on a water-bath and then spontaneously, followed by drying at 100°, leaves not more than 0.002% *w/v* of residue.

A British Standard Specification (B.S.S. No. 575—1934) has been issued by the British Standards Institution for carbon tetrachloride. The specification includes requirements regarding description, specific gravity, distillation-range, residue on evaporation, acidity, oxidisable impurities, free chlorine, sulphur

compounds and sampling, and the appendices describe the methods and apparatus to be used.

The proportion of carbon disulphide in benzene, carbon tetrachloride and other liquids can be found colorimetrically by means of diethylamine and a copper salt. One part in 1,000,000 parts of benzene can be detected.—T. Callan, J. A. R. Henderson and N. Strafford, *J. Soc. chem. Ind., Lond.*, 1932, 193.

Tentative methods for the determination of carbon tetrachloride in mixtures or capsules are described in *Methods of Analysis (A.O.A.C., 1940, 603)*.

Hexachlorethane. A British Standard Specification (*B.S.S. No. 577—1934*) has been issued by the British Standards Institution for hexachlorethane. The specification includes requirements regarding description, melting-range (183° to 187°), moisture, matter insoluble in alcohol, ash, acidity and alkalinity, grading and sampling, and the appendices describe the methods and apparatus to be used.

Diphenanum (B.P.C. Supp. III). $C_{14}H_{13}O_2N = 227.1$. Contains not less than 99.5% of the pure substance, determined by digestion with 50% nitrogen-free sulphuric acid and distillation of the ammonia produced. M.p., 146° to 150° . Loss at 100° , not more than 0.2%. Ash, not more than 0.1%.

Iodoformum (B.P.). $CHI_3 = 393.8$. Should contain 99% of CHI_3 . Assayed by digestion overnight with alcohol (95%), N/10 silver nitrate and nitric acid, dilution, and back titration with N/10 ammonium thiocyanate. M.p., 120° to 122° . Ash limit, 0.2%. The *N.F. VII* specifies that Iodoformum should lose not more than 1% on drying over sulphuric acid for 24 hours, and leave not more than 0.2% of ash. Iodoformum, *P. Helv. V*, is assayed by a methyl alcohol and silver nitrate process similar to that following; it contains 99.3% of CHI_3 .

Assay. The following assay for iodoform is recommended by the Subcommittee on General Organic Chemicals of the Committee on General Chemistry:—Dissolve about 0.5 g., accurately weighed, in 50 ml. of alcohol (95%) and add 50 ml. of N/10 silver nitrate and 5 ml. of nitric acid. Boil under a reflux condenser for thirty minutes, cool, add 150 ml. of water, and titrate with N/10 ammonium thiocyanate, using solution of ferric ammonium sulphate as indicator. Each ml. of N/10 silver nitrate, precipitated by the iodide formed, is equivalent to 0.01313 g. of CHI_3 .—(*British Pharmacopœia Commission Report*, No. 14, September 1939).

An official method for the determination of iodoform in iodoform ointment is described in *Methods of Analysis (A.O.A.C., 1940, 619)*.

Carbasus Iodoformi (B.P.C.). The iodoform, extracted with alcohol and titrated as Iodoformum, should be equivalent to from 4 to 6%.

CHROMII TRIOXIDUM

Chromii Trioxidum (B.P.). $CrO_3 = 100.0$. Contains not less than 95% of CrO_3 . Determined by oxidation of potassium iodide in solution, acidified with dilute sulphuric acid, and titration with N/10 sodium thiosulphate, using starch mucilage as indicator. Water-soluble matter after ignition, not more than 2%. The *U.S.P. XII* requires a purity of 98%, using hydrochloric acid to acidify the titration liquid.

Potassii Dichromas (B.P.C.). $K_2Cr_2O_7 = 294.2$. Assayed with potassium iodide and N/10 sodium thiosulphate, it contains not less than 99% of pure salt.

CHRYSAROBINUM

Chrysarobinum (B.P.) Ash, not more than 0.5%. The U.S.P. XII fixes an ash limit of 0.3%.

Dithranol (B.P. Add. VI). $C_{14}H_{10}O_3 = 226.1$. M.p., 174° to 178° . Ash, not more than 0.1%. Loss at 100° , not more than 1%. A test for absence of dihydroxanthraquinone is included.

CINCHONA

(with CINCHONA ALKALOIDS)

Cinchona (B.P.). Contains not more than 2% of other organic matter, and not less than 6% of total alkaloids of which not less than one half is quinine and cinchonidine. Ash, not more than 4%. *Assay* (B.P. '32 process): The powdered bark is mixed with strong solution of lead subacetate and water and allowed to stand; after standing with ammoniacal alcohol, it is subjected to continuous extraction with more ammoniacal alcohol; the alcohol is recovered, and the residue treated with successive portions of sulphuric acid and water until completely extracted; the filtered aqueous shakings, cleaned by shaking with chloroform, are made alkaline with ammonia and the alkaloid extracted with chloroform, dried at 100° and weighed. The total alkaloids are then dissolved in water, N/1 sulphuric acid and alcohol, and the boiling liquid made just pink to hæmatoxylin with N/10 sodium hydroxide. The acidified and filtered boiling liquid is concentrated, sodium potassium tartrate added, and set aside for 24 hours to crystallise; the alkaloidal tartrates are decomposed with sodium hydroxide and finally extracted with chloroform, evaporated, and the residue of quinine and cinchonidine dried at 100° and weighed. Cinchona, N.F. VII, should contain not less than 5% of alkaloids, determined by heating with hydrochloric acid and water, cooling, macerating with ether, chloroform and stronger ammonia solution; followed by extraction of an aliquot part with 5% sulphuric acid, finally rendering ammoniacal and extracting with chloroform.

Quinquina Jaune, Fr. Cx. 1937, contains not less than 6% of total alkaloids and Quinquina Rouge, not less than 5%. Assayed by digestion with dilute hydrochloric acid and water, shaking with soda and ether-chloroform and clarifying with tragacanth, evaporating an aliquot and titrating with N/10 hydrochloric acid to methyl red.

Cortex Chinæ, P.G. VI, is the bark of *Cinchona succirubra* Pavon, and should yield not less than 6.5% of alkaloid by the process prescribed. Cortex Cinchonæ, P. Helv. V, is the bark of cultivated *Cinchona succirubra* Pavon, containing not less than 6.5% of alkaloids; and yields not more than 6% of ash and not less than 15% of water-soluble extractive.

Percolation of a moderately fine powder (44/85) gave better extraction of both alkaloids and total solids, when compared with percolation of either a fine powder (85) or a moderately coarse powder (22/60). This indicates that an optimum degree of comminution exists for percolation of cinchona. The

relative percentage of alkaloids to other solids increases in successive fractions of percolate.—A. W. Bull, *Quart. J. Pharm.*, 1935, 378.

For a comparison of the results given by the B.P. assay process with those given by the methods of some other pharmacopœias see P. A. W. Self and C. E. Corfield, *Quart. J. Pharm.*, 1930, 410. The B.P. assay processes for cinchona galenicals are based on a further paper by the same authors (*Quart. J. Pharm.*, 1931, 335).

A method of extraction has been suggested in which the drug is percolated with boiling alcohol, first in the presence of potassium hydroxide then during the addition of alcoholic hydrochloric acid and finally in the presence of excess of hydrochloric acid. The extraction is said to be complete in two hours. The results for total alkaloids are usually lower than by the official method, but it is claimed that this is due to obtaining a purer residue of alkaloids.—N. L. Allport and D. Friend, *Quart. J. Pharm.*, 1938, 450.

Alpha-Naphthol Test for Cinchona Alkaloids. Added to an aqueous solution of quinine sulphate, a few drops of fresh saturated alcoholic alpha-naphthol solution to which a few drops of concentrated sulphuric acid (2 drops per ml.) have been added, produce a yellow precipitate; when the reagent is in excess a yellow solution results. Quinidine, cinchonidine and cinchonine sulphates act likewise; no other white alkaloids appear to give this reaction. Cinchona alkaloids can thus be detected in presence of atropine, morphine, cocaine, strychnine, caffeine, brucine, codeine and antipyrine. A drop of the reagent added to chloroform or ether residues of any of the cinchona alkaloids gives a yellow colour.—Watson, *Amer. J. Pharm.*, 1913, 502; *Pharm. J.*, ii/1913, 881.

"Grey" cinchona bark from Huanuco found to contain quinine 0.45%, cinchonidine 0.22%, cinchonine 0.63%, amorphous alkaloid 0.48%.

A further sample of S. American bark contained 5.49% of cinchonine and only 0.027% of quinine. It consisted of *C. nitida* and other varieties, and was also "grey" bark. The abnormal content of cinchonine probably due to cultivation or growth at low altitudes and in hot moist atmosphere.—B. F. Howard and O. Chick, *Yearb. Pharm.*, 1920, 385.

Approximate determination as used by the planters for *C. Ledgeriana*. Extract with ether, using slaked lime and sodium hydroxide. Dissolve residue in ether and N/HCl, and titrate with N/NaOH, using litmus. Precipitate tartrates of quinine and cinchonidine, filter, wash, dry and weigh. Determine optical rotation, α , and calculate quantity of quinine and cinchonidine from Cammellin's table. To the filtrate from the tartrates add NaI solution to precipitate quinidine, cinchonine and amorphous alkaloids. Separate quinidine with 94% alcohol.—*Yearb. Pharm.*, 1923, 8.

Extractum Cinchonæ (B.P.). Determined by dilution with alcohol and water, extraction of the acidified liquid with chloroform, making alkaline with sodium hydroxide solution, extraction with chloroform, finally drying at 100° and weighing. Contains 9.5 to 10.5% of alkaloids.

Extractum Cinchonæ Liquidum (B.P.). Contains 4.75 to 5.25% w/v of alkaloids of Cinchona.

Tinctura Cinchonæ (B.P.). Contains 0.95 to 1.05% w/v of alkaloids of Cinchona.

Tinctura Cinchonæ Composita (B.P.). Contains 0.475 to 0.525% w/v of alkaloids. The N.F. VII tincture contains 0.4 to 0.5% w/v of alkaloids of cinchona. Assayed by absorption and shaking with ether-chloroform (4:1) and ammonia solution, followed by extraction of an aliquot with dilute acid, finally transferring to chloroform with ammonia and weighing the alkaloid after evaporation of the solvent.

Tinctura Cinchonæ Composita Concentrata (B.P. Add. V). Determined by the process for Extractum Cinchonæ after evaporation, it contains 1.9 to 2.1% w/v of alkaloids of Cinchona.

Æthylhydrocupreinae Hydrochloridum (B.P.C.).

$C_{21}H_{28}O_2N_2 \cdot HCl = 376.7$. By extraction with chloroform from ammoniacal solution, evaporation, and drying at 100° , it contains not less than 90% of $C_{21}H_{28}O_2N_2$. The *N.F. VII* substance also contains 90% of the base. Æthylhydrocupreinum basicum and Æthylhydrocupreinum hydrochloridum are official in the *P. Helv. V* and each contains not less than 99% of the pure substance.

Cinchonidinae Sulphas (B.P.C.). $(C_{19}H_{22}ON_2)_2 \cdot H_2SO_4 \cdot 7H_2O = 812.6$. Loss at 100° , not more than 16%. Ash, not more than 0.1%. A limit test for quinine, cinchonine and quinidine is described. The salt of the *N.F. VII* is the trihydrate which should lose not more than 12% at 100° .

Cinchonidine sulphate, though very soluble, forms a double compound with quinine sulphate and so always crystallises with the quinine to some extent.—G. E. Shaw, Streatfeild Memorial Lecture, 1934.

Cinchoninae Hydrochloridum (B.P.C.). $C_{19}H_{22}ON_2 \cdot HCl$, $2H_2O = 366.7$. Loss at 100° , not more than 10%. Ash limit, 0.1%.

Cinchoninae Sulphas (N.F. VII). $(C_{19}H_{22}ON_2)_2 \cdot H_2SO_4 \cdot 2H_2O = 722.9$. Loss at 100° , not more than 5%.

Quinetum (B.P.C.). Yields not less than 60% of quinine and cinchonidine, and loses not more than 5% at 100° . Ash, not more than 1%. Assayed by the *B.P.* method for Totaquina.

The Malaria Commission of the League of Nations recommended (1932) that quinetum should be the name applied to a mixture of quinine, cinchonidine and cinchonine in equal parts, this being approximately the proportion in which the alkaloids occur in *Cinchona succirubra*. Of three commercial samples, only one met this specification.—J. A. Goodson and T. A. Henry, *Quart. J. Pharm.*, 1932, 161.

Totaquina (B.P.). Should contain not less than 70% of crystallisable alkaloids of which at least one-fifth is quinine. On drying for one hour at 70° and then at 100° , the loss is not more than 5%. Ash, not more than 5%. *Assay (B.P. '32 process)*: a boiling solution of 2 g. in 20 ml. of N/1 sulphuric acid, 40 ml. of water and 40 ml. of 95% alcohol, made just alkaline to litmus with N/10 sodium hydroxide, is cooled and made just acid, boiled and filtered; the filtrate is evaporated to 120 g., 30 g. of sodium potassium tartrate added and stood for 24 hours. The precipitate, collected on a hardened filter, is washed with 80 ml. of 25% sodium potassium tartrate solution, decomposed with sodium hydroxide and extracted with chloroform; the proportion of quinine in this residue of quinine and cinchonidine is estimated by a methoxyl determination, by interaction with hydriodic acid, passing the methyl iodide into silver nitrate and weighing as AgI. The filtrate and washings from the precipitated tartrate are made alkaline with sodium hydroxide and extracted with ether, the alkaloid transferred to acid and the cinchonine precipitated by running into ether and N/1 sodium hydroxide; the precipitate, collected on a weighed filter, is washed with the ether from the filtrate and with two further ether shakings of the aqueous solution, and finally dried at 100° , adding a correction for solubility in ether. The separated ethereal layer of the filtrate is extracted

with 10% *w/w* solution of glacial acetic acid, the boiling solution neutralised with dilute ammonia, 5 g. of potassium iodide added and stood overnight. The precipitate, washed with 50% alcohol, is dried at 100°, weighed as quinidine hydriodide and corrected for loss due to solubility. The *U.S.P. XII* substance contains not less than 10% of anhydrous quinine, not less than 25% of cinchonidine and anhydrous quinine combined, and a total of not less than 70% of cinchonidine, cinchonine, anhydrous quinidine, and quinine. Ash, not more than 6%. *Totaquina, Fr. Cx.* 1937, contains not less than 70% of crystallisable alkaloids of which not less than 15% is quinine and not more than 20% is amorphous alkaloids; ash, not more than 5%; loss at 100°, not more than 5%. Assayed for quinine and cinchonidine by precipitation with sodium potassium tartrate (a solubility correction is added), the proportion of quinine being found by methoxyl determination. For the determination of cinchonine the alkaloid, recovered from the tartrate filtrate, is dissolved in 15 to 25 ml. of alcohol (96%) diluted with an equal volume of water and after 24 hours filtered and dried at 105° (correction for each ml. alcohol = 0.001 g.). Quinidine is precipitated as iodide (correction = 0.0008 g. for each ml. alcohol).

Assay of Totaquine. In the *U.S.P. XII* assay, quinine and cinchonidine are separated as tartrates which are dried and weighed. Quinine is determined in the precipitate by dissolving 0.5 g. in 3.8 ml. of N/1 hydrochloric acid and sufficient distilled water to produce 25 ml. The solution is slowly inverted 3 times with 20 mg. of activated charcoal and filtered immediately through a dry filter into a dry flask. The first few ml. are rejected and the optical rotation of the filtrate is determined in a 200 mm. tube at 25° using sodium light. The percentage of anhydrous quinine is calculated from the following formula:—

$$\frac{(M - 322) \times 0.79 \times T}{2.02 \times S}$$

M = observed angular rotation *n* minutes, T = weight of dried tartrate precipitates and S = weight of totaquine taken for the assay. The separation of cinchonine and quinidine is essentially the same as that in the *B.P.* process.

Alternative assay processes for *Totaquina* Type I (made direct from the bark of *C. succirubra*) and Type II (made from residues of quinine extraction and adjusted by the addition of quinine) are described.—*Quart. Bull. Hlth Org. L. o. N.*, 1934, 3, 339.

A tentative method for determination of cinchona alkaloids is described in *Methods of Analysis (A.O.A.C., 1940)*. The total alkaloids (about 0.5 g.) dissolved in 50 ml. of 0.225N sulphuric acid are heated on a steam-bath for 10 minutes, 5% sodium hydroxide added until a faint permanent precipitate is produced, sufficient 0.225N sulphuric acid to clear and a further 5 ml. added and then 25 ml. of a Rochelle salt solution (100 ml. of saturated solution with 3 ml. of 0.225N sulphuric acid). After allowing to remain at 10° to 15° for 2 hours, with occasional stirring, the precipitate is washed with 40 ml. of Rochelle salt solution and water (equal parts). The tartrates are decomposed with warm 10% sulphuric acid and extracted with ammonia and chloroform. The proportions of quinine and cinchonidine in the dried residue are determined by dissolving in 0.225N sulphuric acid (1 ml. for each 0.015 g. of alkaloid) and reading the angular rotation produced, using the longest tube possible and a bichromate filter. The proportion of quinine (Q) is calculated from the formula, $Q = (-68.44) (a + 2.7)$; *a* = the observed rotation in a 100 mm. tube. For quinidine the acid tartrate solutions are heated on a steam-bath for 10 minutes, 0.5 g. of potassium iodide added and kept at 10° to 15° for 2 hours. The precipitate is washed with 15 ml. of ice water and dried at 100° for 1 hour. The filtrate and washings made ammoniacal are extracted with chloroform and the cinchonine dried at 100° for 1 hour.

Reaction for Distinguishing Quinine from Quinidine. Inasmuch as quinine and quinidine are optical isomers, most of their reactions are identical. Thirty commonly used alkaloidal reagents give the same test with both. The differentiating test is as follows: To a solution of the pure alkaloid in very dilute sulphuric acid add a drop of $KI \cdot I_2$ solution, shake, and dilute, if the precipitate is heavy, until a light translucent suspension is obtained. In the case of quinine the precipitate appears dark brown while if the sample is quinidine it will appear yellow.—L. Rossi and J. A. Sozzi, per *Amer. J. Pharm.*, 1935, 491.

Quinidina (B.P.C.). $C_{20}H_{24}O_2N_2 \cdot 2H_2O = 360.2$. Loss at 100° , not more than 10%. Ash, not more than 0.1%.

Quinidinæ Sulphas (B.P.). $(C_{20}H_{24}O_2N_2)_2 \cdot H_2SO_4 \cdot 2H_2O = 782.5$. Loss at 120° , not more than 5%. Ash, not more than 0.04%. It is tested for other cinchona alkaloids by precipitating as iodide and adding ammonia to the filtrate, when no turbidity should be produced. The *U.S.P. XII* allows the same moisture limit and 0.1% of ash; 0.2 g. complies with a test for carbonisable substances.

Chinidinum sulfuricum, *P. Helv. V*, should contain 99.5% of $(C_{20}H_{24}O_2N_2)_2 \cdot H_2SO_4 \cdot 2H_2O$. Other cinchona alkaloids are excluded by the following tests:—(a) Dissolve 5 g. of quinidine sulphate in 10 ml. of water, add 0.5 g. of potassium iodide and maintain the liquid for 1 hour at 15° , shaking strongly and frequently; filter through a glass filter and add 2 drops of 3.4% *w/v* solution of ammonia; no cloudiness should be produced either immediately or within 1 hour. (b) The rotation of a solution of 0.391 g. in 0.8 ml. of 7.3% *w/v* hydrochloric acid and 20 ml. of water, at 20° and in a 200 mm. tube, should be between $+10.5^\circ$ and $+10.7^\circ$.

Tabellæ Quinidinæ Sulphatis (U.S.P. XII). Contain 94 to 106% of the labelled amount of $(C_{20}H_{24}O_2N_2)_2 \cdot H_2SO_4 \cdot 2H_2O$, including all tolerances. Assayed by macerating a weighed quantity of powdered tablets with water and dilute sulphuric acid overnight, filtering, making alkaline an aliquot of the filtrate with ammonia solution, extracting with chloroform, filtering the combined chloroform extracts, removing the chloroform, evaporating the residue with alcohol and drying at 100° .

QUININE AND ITS SALTS

The following table shows the principal *B.P.* and *B.P.C.* standards for quinine and the salts of quinine. All, with the exception of Quininæ et Æthylis Carbonas, are required to comply with the *B.P.* test for limit of other cinchona alkaloids. The quantities which are directed to be taken for the test are given in the fourth column of the table. The specified quantity of the substance with 50 ml. of water and 5 ml. of dilute sulphuric acid is made ammoniacal with 5 ml. of dilute solution of ammonia and extracted with 30 ml. and then 20 ml. of chloroform, each being washed with two 10 ml. quantities of water; most of the chloroform is evaporated, about 3 ml. of dehydrated alcohol added and quickly evaporated. The opaque residue is dissolved in 20 ml. of alcohol and 20 ml. of water, and 1 ml. of a methyl red solution added; at 75° the solution is adjusted with *N/5* sulphuric acid to the same colour as 56 ml. of solution of *pH* 5.44 at 20° , with 1 ml. of the methyl red solution; the mixture is evaporated to

| Substance | Percentage of Anhydrous Quinine | Maximum % loss on drying | Quantity to be taken in "other cinchona alkaloids" test | Maximum % of ash |
|--|---------------------------------|-------------------------------|--|------------------|
| Quinina (<i>B.P.C.</i>) $C_{20}H_{24}O_2N_2 \cdot 3H_2O = 378.3$ | not less than 85 | 15 at 100° | 1.1 g. (in 20 ml. of 90% alcohol commencing with addition of 20 ml. of water and methyl red) | 0.05 |
| Quininæ Acetylsalicylas (<i>B.P.C.</i>) $C_{20}H_{24}O_2N_2 \cdot C_8H_8O_4 = 504.3$ | not less than 63.5 | 1 at 100° | 1.5 g. | 0.05 |
| Quininæ Arsenas (<i>B.P.C.</i>) $(C_{20}H_{24}O_2N_2)_3 \cdot H_3AsO_4 \cdot 8H_2O = 934.5$ | not less than 69 | 16 at 100° | 1.4 g. | — |
| Quininæ Benzozas (<i>B.P.C.</i>) $C_{20}H_{24}O_2N_2 \cdot C_6H_5O_2 = 446.3$ | 72 to 75 | — | 1.4 g. | 0.1 |
| Quininæ Bisulphas (<i>B.P.</i>) $C_{20}H_{24}O_2N_2 \cdot H_2SO_4 \cdot 7H_2O = 548.4$ | about 59 | 24 at 110° | 1.7 g. (in 50 ml. water without addition of acid) | 0.04 |
| Quininæ Citras (<i>B.P.C.</i>) $(C_{20}H_{24}O_2N_2)_3 \cdot C_6H_5O_7 \cdot 7\frac{1}{2}H_2O = 1800$ | not less than 74.5 | 10.5 at 100° | 1.3 g. | 0.1 |
| Quininæ Dihydrobromidum (<i>B.P.C.</i>) $C_{20}H_{24}O_2N_2 \cdot 2HBr \cdot 3H_2O = 540.1$ | not less than 59 | 11 at 100° | 1.7 g. | 0.1 |
| Quininæ Dihydrochloridum (<i>B.P.</i>) $C_{20}H_{24}O_2N_2 \cdot 2HCl = 397.1$ | about 81 | 3 at 110° | 1.2 g. | 0.04 |
| Quininæ Disalicylsolicylas (<i>B.P.C.</i>) $C_{20}H_{24}O_2N_2 \cdot C_{18}H_{16}O_{10} = 840.4$ | 38 to 40 | — | 2.5 g. | — |
| Quininæ et Ethylis Carbonas (<i>B.P.</i>) $C_{21}H_{21}O_2N_2 \cdot CO_2 \cdot C_2H_5 = 396.2$ | — | 2 over H_2SO_4 for 24 hours | — | 0.04 |
| Quininæ et Ureæ Hydrochloridum (<i>B.P.C.</i>) $C_{20}H_{24}O_2N_2 \cdot CH_4N_2O_2 \cdot 2HCl \cdot 5H_2O = 547.3$ | not less than 58 | 16.5 at 100° | 1.7 g. | 0.1 |

| Substance | Percentage of Anhydrous Quinine | Maximum % loss on drying | Quantity to be taken in other cinchona alkaloids' test | Maximum % of ash |
|---|---------------------------------|--------------------------|--|------------------|
| Quininæ Glycerophosphas (B.P.C.) (C ₂₀ H ₃₄ O ₄ N ₂), C ₂ H ₅ O ₄ P, 4H ₂ O = 892.6 | not less than 70 | 8.5 at 100° | 1.4 g. | — |
| Quininæ Hydriodidum (B.P.C.) C ₂₀ H ₃₄ O ₄ N ₂ ·HI = 452.1 | not less than 71 | 1 at 100° | 1.4 g. | 1.0 |
| Quininæ Hydrobromidum (B.P.C.) C ₂₀ H ₃₄ O ₄ N ₂ ·HBr, 2H ₂ O = 441.2 | not less than 73 | 9 at 100° | 1.3 g. | 0.1 |
| Quininæ Hydrochloridum (B.P.) C ₂₀ H ₃₄ O ₄ N ₂ ·HCl, 2H ₂ O = 396.7 | about 82 | 10 at 110° | 1.2 g. | 0.04 |
| Quininæ Hypophosphis (B.P.C.) C ₂₀ H ₃₄ O ₄ N ₂ ·H ₃ PO ₃ , 2H ₂ O = 426.3 | not less than 74 | 9 at 100° | 1.3 g. | — |
| Quininæ Lactas (B.P.C.) C ₂₀ H ₃₄ O ₄ N ₂ ·C ₃ H ₅ O ₂ = 414.3 | not less than 72 | 3 at 100° | 1.3 g. | 0.1 |
| Quininæ Phosphas (B.P.C.) (C ₂₀ H ₃₄ O ₄ N ₂) ₃ ·2H ₃ PO ₄ , 6H ₂ O = 1277 | 74 to 78 | 10 at 100° | 1.3 g. | — |
| Quininæ Salicylas (B.P.C.) C ₂₀ H ₃₄ O ₄ N ₂ ·C ₇ H ₅ O ₃ ·H ₂ O = 480.3 | not less than 67 | 4 at 100° | 1.4 g. | 0.1 |
| Quininæ Sulphas (B.P.) (C ₂₀ H ₃₄ O ₄ N ₂) ₃ ·H ₂ SO ₄ ·7½H ₂ O = 881.6 | about 73.5 to 76.5 | 11 to 16 at 100° | (On the substance dried at 50° for 2 hours commencing with 1 g. boiled with 30 ml. of water) | 0.04 |
| Quininæ Tannas (B.P.) | 30 to 35 | 10 at 100° | 3.3 g. (after preliminary treatment) | 0.3 |
| Quininæ Valerianas (B.P.C.) C ₂₀ H ₃₄ O ₄ N ₂ ·C ₄ H ₁₀ O ₃ ·H ₂ O = 444.3 | not less than 71 | — | 1.4 g. | 0.1 |

dryness on a water-bath and powdered, 1 g. boiled with 30 ml. of water under a reflux condenser, is cooled rapidly to 15°, shaking vigorously, and maintained at that temperature, with frequent shaking, for 30 minutes, and filtered rapidly. 6.5 ml. of solution of ammonia (10.0 to 10.2% *w/w* NH_3) at 15° is added all at once to 5 ml. of the clear filtrate at 15° and on mixing gently a clear liquid should be produced. Quininæ Tannas is extracted, before applying this test, with ether from a sodium hydroxide, ether and tragacanth mixture, the ethereal liquid being then extracted with sulphuric acid.

Quininæ Sulphas, *U.S.P. XII*, is the dihydrate. The moisture, determined at 100°, is not more than 5%. The test for "other cinchona alkaloids" described for the sulphate and used, after preliminary treatments, for the other *U.S.P. XII* quinine salts, differs slightly in detail from the *B.P.* test. 1.8 g. of the salt dried at 50° is shaken for 30 minutes with 20 ml. of water at 65°, macerated for 2 hours at 15° and filtered; 5 ml. of the filtrate produces a clear mixture with 6 ml. of ammonia solution (10 to 10.2%). 0.2 g. of quinine salts complies with the test for readily carbonisable substances except in the case of Quininæ Hydrochloridum *U.S.P. XII*, where the amount used is 0.1 g. and 2 ml. of sulphuric acid is used in place of 5 ml.

Chininum sulfuricum, *P. Helv. V*, is the stable dihydrate, $(\text{C}_{20}\text{H}_{24}\text{O}_2\text{N}_2)_2 \cdot \text{H}_2\text{SO}_4 \cdot 2\text{H}_2\text{O}$, and contains from 4.5 to 4.7% of water. The absence of other cinchona alkaloids is ensured by the following tests:—(a) Dissolve 0.85 g. of the quinine sulphate in 50 ml. of boiling water in a tared flask; cool rapidly and shake well, and make up to 51 g. with water; add 5 g. of powdered potassium sulphate and keep at 20° for 30 minutes, shaking frequently and strongly. Filter through a porous glass filter, mix 20 ml. of the filtrate with 6.0 ml. of water, add 1 drop of 8.5% *w/v* sodium hydroxide solution and shake vigorously. No turbidity is produced immediately or within one minute. (b) The rotation of a solution of 0.746 g. in 1 ml. of 9.8% *w/v* sulphuric acid and 1 ml. of 7.3% *w/v* hydrochloric acid with sufficient water to make 20 ml., at 20° and in a 200 mm. tube should be between -17.8° and -18° .

Other cinchona alkaloids are excluded from Chininum dihydrochloricum, *P. Helv. V*, and from Chininum hydrochloricum, *P. Helv. V*, by dissolving 0.6 g. of the acid salt in 7.5 ml. of boiling water and neutralising with 1% ammonia to methyl red, or 0.5 g. of the hydrochloride in 15 ml. of boiling water, then adding 6 ml. of potassium chromate solution, cooling, shaking well and filtering, when on adding 2 drops of dilute sodium hydroxide solution to the filtrate no change occurs (quinidine); after warming for 1 hour on a water-bath and then standing for 24 hours the solution remains clear (cinchonidine). The angular rotation of 0.794 g. of the dried acid salt in 2 ml. of dilute sulphuric acid and water to 20 ml. in a 200 mm. tube or of 0.721 g. of the hydrochloride is -17.8° to -18.0° (other cinchona alkaloids).

The monohydrate is the *Quininæ Hydrobromidum* of the *N.F. VII*; it should lose not more than 5% at 100°. In the *Fr. Cx.* 1937 the test for other alkaloids in quinine sulphate is carried out as follows:—Dissolve, by refluxing 1 g. in 34 ml. of water, cool rapidly to 15°, maintain at this temperature, with frequent shaking, for 30 minutes and filter; 5 ml. with 5 ml. of dilute ammonia remains clear for 24 hours. 5 ml. of the filtrate evaporated and dried at 100°, does not weigh more than 0.008 g. Also 0.5 g. dried at 100°, dissolved in 20 ml. water with 2.5 ml. N/10 sulphuric acid has a α_D of -240° . Quinine sulphate is the dihydrate and should lose 4 to 5.1% at 110°.

Tabellæ Quininæ Sulphatis (U.S.P. XII). Contain 94 to 106% of the labelled amount of $(C_{20}H_{24}N_2O_3)_2 \cdot H_2SO_4 \cdot 2H_2O$, including all tolerances. Assayed by the method described for quinidine sulphate tablets.

Quininæ et Æthylis Carbonas (B.P. Add. I). M.p. not below 90°. Loss over sulphuric acid for 24 hours, not more than 2%. Ash, not more than 0.04%. Practically all commercial samples have a melting-point of about 89° to 91° after drying over sulphuric acid, but it is difficult to obtain consistent results unless a standard method of procedure is adopted. *Quininæ Æthylcarbonas*, *U.S.P. XII*, has a m.p. of 89° to 91°.

Quininæ Phosphas (N.F. VII) has 5 molecules of water of crystallisation and yields 74 to 78% of anhydrous quinine.

Liquor Quininæ Ammoniatas (B.P.). Contains 1.9 to 2.1% w/v of Quinine Sulphate and 0.9 to 1.05% w/v of NH_3 . Quinine is determined by extraction with chloroform and the ammonia is titrated directly with N/2 hydrochloric acid using methyl red indicator.

For a method for the extraction of quinine from viscera, see under *Morphina*, p. 307.

Tanret's Reagent. Potassium iodide 3.32 g., mercuric chloride 1.35 g., acetic acid 20 ml., diluted with distilled water to 60 ml. Precipitates alkaloids and albumin. Better than Mayer's for quinine test in urine, providing urine is albumin-free.

Determination by Absorption Spectrophotometry. Quinine salts have a strong absorption band in the near ultra-violet and the transmission curve of a solution of quinine in dil. hydrochloric acid shows maximum absorption at ca. 340 m μ . Of the compounds used with quinine in medications, strychnine, acetanilide, acetylsalicylic acid, camphor, phenolphthalein, caffeine, most blue, green and red dyes, glycerin, alcohol and sugars have no absorption bands near 340 m μ and quinine may be determined directly in the presence of these. Solutions of ferric salts absorb strongly in this region, but the colourless complex of the ferric ion and phosphate ion has no absorption band in this region.—J. Carol, *Analyst*, 1942, 299.

Mepacrinæ Hydrochloridum (B.P. Add. VI).

$C_{23}H_{30}ON_3Cl \cdot 2HCl \cdot 2H_2O = 508.7$. Loses from 6 to 8% at 130° and then contains not less than 99.0% of the pure substance. Determined by extraction with chloroform from a solution made alkaline with sodium hydroxide and drying at 100°. Limit of 2-chloro-7-methoxyacridone, 0.05%. *Quinacrinæ Hydrochloridum*, *U.S.P. XII*, assayed by precipitation with excess N/10 potassium dichromate from a solution containing sodium acetate and glacial acetic acid, filtration and determination of the excess N/10 potassium dichromate iodometrically in an aliquot of the filtrate, contains 77 to 80.2% of anhydrous base, corresponding

to not less than 98% of $C_{23}H_{30}ClN_3O \cdot 2HCl \cdot 2H_2O$. Loss on drying at 105° , 6 to 8%; pH of 1% solution, about 5.

Tabellæ Quinacrinæ Hydrochloridi (*U.S.P. XII*). Contain 95 to 110% of the labelled amount of $C_{23}H_{30}ClN_3O \cdot 2HCl \cdot 2H_2O$, including all tolerances. In the assay, a weighed quantity of powdered tablets is triturated twice with ether and the ether extracts discarded. Solution of ammonia is added to the residue which is then extracted completely with ether. To the combined ether extracts, glacial acetic acid is added and the ether removed. The residue is treated with water, sodium acetate and glacial acetic acid and the assay completed as described for quinacrine hydrochloride.

Mepacrinæ Methanosulphonas (*B.P. Add. VI*).

$C_{23}H_{30}ON_3Cl \cdot 2CH_3SO_3H \cdot H_2O = 591.9$. Contains not less than 99.0% of the pure substance, calculated with reference to the substance dried at 130° . The filtrate from shaking with 40 parts of anæsthetic ether shows no greater fluorescence than a solution of 0.25 mg. of chloromethoxyacridone in 100 ml. of anæsthetic ether. Loses from 2 to 4% at 130° .

Pamaquinum (*B.P. Add. IV and VI*). $C_{42}H_{45}O_7N_3 = 703.4$. The anhydrous substance yields 43 to 45% of 6-methoxy-8-(ω -diethylamino- α -methylbutyl)-aminoquinoline, by extraction with benzene from sodium hydroxide solution and drying at 100° for one hour in an atmosphere of nitrogen; and by precipitation from the alkaline solution with hydrochloric acid and subsequent drying at 105° , 53 to 57% of 2:2'-dihydroxy-1:1'-dinaphthylmethane-3:3'-dicarboxylic acid. A limit for 6-methoxy-8-aminoquinoline is included. Loss at 100° for 2 hours, not more than 4%. Residue on ignition with sulphuric acid, not more than 0.2%. Pamaquinæ Naphthoas, *U.S.P. XII*, yields not more than 1% ash and otherwise complies with the same standards for purity. Assayed for the acid by acidification of a solution with dilute hydrochloric acid and allowing the mixture to stand overnight. The precipitate is then collected on a tared filtering crucible, washed and dried at 100° . Pamaquin base is determined in the combined washings and filtrate by cooling to 15° , adding crushed ice, and slowly titrating M/10 sodium nitrite until the titrated solution, after being allowed to stand 1 minute, gives a blue colour with a smear of starch-iodide paste, used as an external indicator. 1 ml. M/10 sodium nitrite = 0.03154 g. of pamaquin base.

Biological Test for Antimalarial Remedies. Numerous substances have been introduced for the treatment of malaria such as mepacrine and pamaquin, and their value is determined in the first place by examining their effect in bird malaria.

The method of carrying out the biological test is as follows:—Canaries are infected, the heads of the infected birds are cut off, and the blood is filtered through glass wool into 1% citrate saline, so that the blood becomes diluted 5 or 10 times. This dilution is kept at 37° and injected into normal birds within 25 minutes. The injection is made into the pectoral muscles. If the dilution of blood is not kept at 37° the appearance of parasites is later in those birds which are last inoculated. The average time of the appearance of parasites in untreated birds varies from 4 to 8 days. (See G. A. H. Burtle, T. A. Henry and J. W. Trevan, *Biochem. J.*, 1934, 426.)

To test the efficacy of any unknown preparation, a group of birds is treated with some substance, such as quinine, used as standard, and a second group is treated with the preparation to be tested. The first dose is given about 4 hours after the canary is infected, and subsequent doses once daily

for the next five days; the doses are given orally by means of a thin piece of rubber tubing passed down the gullet. The birds are then observed in order to discover how many days elapse before the parasites appear in their blood; the average time for the appearance of parasites in a group of birds which received the same dose is then determined, and the average time for the unknown preparation is compared with the average time for the standard. Thus, a group of six birds which received a total dose of 30 mg. of quinine, reckoned per 20 g. bird weight, showed parasites after an average of 18.2 days, while a group receiving the same dose of dihydrocinchonidine showed parasites after an average of 9.3 days. Thus dihydrocinchonidine is less efficient than quinine in treating bird malaria.

CINCHOPHENUM

Cinchophenum (B.P.). $C_{16}H_{11}O_2N=249.1$. By titration in warm alcohol with N/1 sodium hydroxide using phenolphthalein indicator, the dried substance contains not less than 99% of $C_{16}H_{11}O_2N$. Loss in a vacuum desiccator over sulphuric acid, not more than 1%. Ash, not more than 0.2%. M.p., 214° to 217° . The *N.F. VII* requires Cinchophenum to contain 99.5% of the pure substance after drying at 100° ; loss at 100° , not more than 2%; ash, not more than 0.25%. Acidum phenylcinolin-carbonicum, *P.G. VI*, melts between 208° and 213° . Acidum phenylcinchoninicum, *P. Helv. V*, m.p. 208° to 210° .

Tentative methods for the determination of cinchophen in the presence of salicylates and of sodium bicarbonate are described in *Methods of Analysis* (A.O.A.C., 1940, 607). In the presence of salicylates, the cinchophen, extracted from the sample with sodium carbonate solution, is dissolved in warm acetic acid, treated with an excess of standard iodine and back-titrated with standard sodium thiosulphate, using starch indicator. When sodium bicarbonate is present, sodium hydroxide is added to the sample to dissolve the cinchophen, the solution is acidified with hydrochloric acid and extracted with chloroform. The residue from evaporation of the chloroformic extract is dissolved in alcohol, and the solution titrated with sodium hydroxide, using phenolphthalein as indicator.

Tabellæ Cinchopheni (*N.F. VII*). Contain 92.5 to 107.5% of the labelled amount of cinchophen including all tolerances. Assayed by preliminary extraction with chloroform, discarding the chloroform, then extracting the residue with neutral alcohol at 65° to 70° , filtering and titrating with N/10 sodium hydroxide using phenolphthalein indicator.

Tabellæ Neocinchopheni (*U.S.P. XII*). Contain 94 to 106% of the labelled amount of $C_{16}H_{11}O_2N$, including all tolerances. Assayed by refluxing twice a weighed quantity of powdered tablets with alcohol, passing the hot alcohol through a filter, finally washing the residue on the filter with small portions of alcohol, then refluxing an aliquot of the alcoholic solution with N/10 alcoholic potassium hydroxide for 1 hour, cooling and titrating the excess alkali with N/10 hydrochloric acid, using phenolphthalein indicator and subtracting from the alkali reading in this titration the figure obtained when a second aliquot of the alcoholic extract of the tablets is mixed with an excess of N/10 alcoholic potassium hydroxide and back titrated with N/10 hydrochloric acid, using phenolphthalein indicator.

CINNAMOMUM

Cinnamomum (B.P.). Ash, not more than 7%. Acid-insoluble ash, not more than 2%. Cinnamomum, *U.S.P. XII*,

from *Cinnamomum Loureirii* Nees, should yield not less than 2.5% *v/w* of volatile oil; foreign organic matter, not more than 2%.

Cortex Cinnamomi, *P.G. VI*, is required to contain not less than 1% of volatile oil. Cortex Cinnamomi ceylanici, *P. Helv. V*, should yield not less than 1.3% of cinnamic aldehyde when assayed by the following process:—Steam distil a mixture of 6 g. of powdered bark and 100 ml. of water, and treat the first 300 ml. of the distillate with a cold solution of 0.25 g. of semioxamazide in 15 ml. of water; shake well for 10 minutes, and allow to stand for at least 20 hours, agitating the mixture occasionally. Collect the precipitate on a tared Gooch filter dried at 150°, wash the precipitate with water, dry for 2 hours at 140° to 150° and weigh after allowing to cool over sulphuric acid. The weight of precipitate, multiplied by 10.14, gives the percentage of aldehyde in the bark. Cortex Cinnamomi chinensis, *P. Helv. V*, must give not less than 1.5% of cinnamic aldehyde.

The Food and Drug Administration of the U.S. Dept. of Agriculture defined cinnamon as the dried bark of cultivated varieties of *Cinnamomum zeylanicum* Nees or of *C. cassia* (L.) Blume from which the outer layers may or may not have been removed, (*Ceylon Cinnamon*), as the dried, inner bark of cultivated varieties of *Cinnamomum zeylanicum* Nees, (*Saigon Cinnamon*), cassia as the dried bark of cultivated varieties of *Cinnamomum cassia* (L.) Blume and *Ground Cinnamon*, ground cassia, as the powder made from cinnamon. Contained not more than 5% total ash and not more than 2% ash insoluble in hydrochloric acid.—*S.R.A., F.D., No. 2, Rev. 5, Nov. 1938.*

The figures for the area of fibres per gramme of powdered cinnamon and cassia bark are 92.5 and 13.1 respectively and hence can be used for the quantitative estimation of cassia in cinnamon or *vice versa*. In addition, the figure for cinnamon varies with the quality of bark and may also be used as a criterion of the quality of the sample.—A. H. Saber, *Quart. J. Pharm.*, 1940, 7.

Artificial cinnamon produced in Germany consists of a mixture of 96% cinnamic aldehyde and 4% eugenic acid which is incorporated in a powder carrier made from pulverised shells of hazelnuts or almonds coloured with a brownish-yellow material. The artificially coloured powder, after drying, is thoroughly mixed with the cinnamic aldehyde-eugenic acid mixture and 0.2% of Ceylon cinnamon oil added to give the fragrance of natural cinnamon.—per *Mfg. Chem.*, 1941, 164.

Oleum Cassiæ (B.P.C.). From *Cinnamomum Cassia* Blume. Assayed by the *B.P.* method for Oleum Cinnamomi should contain not less than 80% *w/w* of aldehydes as C_9H_8O . Sp. gr., 1.055 to 1.065. n_{D20}^{20} , 1.600 to 1.606. Soluble in 2 volumes of alcohol (80%, sp. gr. 0.8634 to 0.8640) and 3 volumes of alcohol (70%, sp. gr. 0.8896 to 0.8901). Oleum Cinnamomi Cassiæ, *P. Helv. V*, contains at least 80% of cinnamic aldehyde; assayed by treatment with water and warm semioxamazide solution and weighing the precipitate after drying at 140° to 150° for 2 hours; 1 g. = 0.6083 g. of cinnamic aldehyde.

Imported oil generally contains lead in excess of the *B.P.C.* limit; it is derived from the leaden vessels in which the oil is received.

Direct determination of total aldehydes in cassia oil. For a method which is stated to avoid the error caused in the ordinary process by the too great excess of the bisulphite reagent, see F. D. Dodge, *Amer. Perfum.*, 1929, 24, 11, per *Quart. J. Pharm.*, 1929, 328.

Seven classes of cassia oil, Saigon, Honan, China, Kwongsai, Korintji and Batavia and Ceylon Cinnamon bark oil, are examined for yield of oil from the bark and for their physical constants, aldehyde content, and acid value and ester value of the non-aldehyde portion. The Ceylon cinnamon bark oil is

characterised by its uniform yield of volatile oil and its lower aldehyde content.—J. F. Clavenger, *J. Ass. agric. Chem.*, 1941, 24, 461-464.

Petroleum Adulteration. Large quantities of imported oils contain this adulterant. If 25 or 50 ml. of the oil is steam distilled and the first 10% collected the oily portion of this distillate will separate into two layers the upper of which will be petroleum, which may be identified by tests.—W. H. Simmons, *Perfum. essent. Oil Rec.*, 1935, 408; C. T. Bennett, *ibid.*, 216.

Oleum Cinnamomi (B.P.). From *Cinnamomum zeylanicum* Nees. Assayed by the B.P. method by interaction with hydroxylamine hydrochloride solution and titration with N/2 potassium hydroxide in 60% alcohol. Cinnamic aldehyde content, 50 to 65% w/w. Sp. gr., 1.000 to 1.030. α_D , 0° to -2°. n_{D20}° , 1.565 to 1.586. Soluble in 3 volumes of 70% alcohol with only a faint opalescence. Oleum Cinnamomi, U.S.P. XII, obtained from cassia, *Cinnamomum Cassia* (Linné) Blume, contains not less than 80% v/v of cinnamic aldehyde; determined by measurement of the oil unabsorbed by saturated sodium sulphite solution, neutralising to phenolphthalein with sodium bisulphite solution, by heating in a water-bath and then heating for a further 15 minutes; sp. gr. at 25°, 1.045 to 1.063; α_D at 25°, +1° to -1°; n_{D20}° 1.6020 to 1.6135. Essence de Cannelle de Ceylan, Fr. Cx. 1937, contains 65 to 75% of cinnamic aldehyde.

The Food and Drug Administration of the U.S. Dept. of Agriculture defined oil of cinnamon, oil of cassia, oil of cassia cinnamon, for food purposes, as the lead-free volatile oil from the leaves or bark of *Cinnamomum cassia* (L.) Blume; it was required to contain not less than 80% v/v of cinnamic aldehyde. Oil of Ceylon cinnamon, the lead-free volatile oil from the bark of the Ceylon cinnamon (*Cinnamomum zeylanicum* Nees), was required to contain not less than 65% w/w of cinnamic aldehyde and not more than 10% w/w of eugenol.—S.R.A., F.D., No. 2, Rev. 5, Nov. 1936.

The aldehyde limits have been restricted in order to eliminate factitious oils containing artificial cinnamic aldehyde and redistilled cassia oil which, although often sold as B.P., lack the true cinnamon oil odour. English distilled oils possess the finest aroma and invariably contain a low percentage of cinnamic aldehyde. Some years ago imported oils frequently contained a large proportion of cinnamon leaf oil but they are no longer marketed.—C. T. Bennett, *Pharm. J.*, i/1933, 150.

Analytical characters which differ from the B.P. but which are more in conformity with true cinnamon oil are given by Gildermeister and Hoffman, *Die Ätherischen Öle*, as follows:—Sp. gr., 1.023 to 1.040; α_D , 0° to -1°, seldom more; n_{D20}° , 1.581 to 1.591; aldehydes, 65 to 75%; eugenol, 4 to 10%; soluble in 2 to 3 volumes of 70% alcohol. Oils containing less than 65% or more than 75% of cinnamic aldehyde are suspect.

Genuine oils frequently fail to dissolve 1 in 3 of 70% alcohol, but are usually soluble in 4 to 5 parts.—Finnemore.

For details of *C. zeylanicum* root, bark, and leaf oils, and *C. cassia* oil, see *Bull. imp. Inst., Lond.*, 1921, 19, 323, per *Quart. J. Pharm.*, 1922, 63.

Powdered cinnamon stored in paper bags lost 11 to 33% of essential oil; stored in glass it lost 0 to 8% during one year.—I. Horváth, per *Quart. J. Pharm.*, 1933, 607.

The physical and chemical constants of cinnamon leaf oil from Uganda, *Quart. J. Pharm.*, 1935, 122, and from the Seychelles, *Quart. J. Pharm.*, 1935, 256. The production of cinnamon bark and cinnamon leaf oils in the Seychelles are described.—Haines, *Perfum. essent. Oil Rec.*, 1936, 6 and 52.

COCAINA

Cocaina (B.P.). $C_{17}H_{21}O_4N = 303.2$. M.p., 97° to 98°. Ash, not more than 0.1%. Tests for absence of isoatropyl-cocaine,

and limit of cinnamyl-cocaine are included. The lower limit for the melting-point in the B.P. is too high, the majority of commercial samples having a melting-point of about 96° to 97° .

Constitutionally, cocaine is ecgonine with the hydrogen atoms in the carboxyl and hydroxyl groups replaced by a methyl and benzoyl group respectively.

Purification of Crude Cocaine. Cocaine, truxilline and cinnamyl-cocaine, being ecgonine derivatives, yield ecgonine, acids, and methyl alcohol on hydrolysis. This fact is of importance commercially as the amorphous residue remaining after extracting as much as possible of the crystalline cocaine can be converted into ecgonine, and this by treatment with benzoic anhydride and methyl alcohol can be converted synthetically into cocaine.

Although formerly care was taken in the extraction to preserve the cocaine, manufacturers now rely on the ecgonine content. After isolation of the crude "cocaine," it is treated so as to reintroduce the methyl and benzoyl groups.

β -Naphthalenesulphonic acid may be used for purifying crude cocaine. Crude cocaine, 10 g., is dissolved in hot water containing 5 g. of the acid and the solution filtered warm. On cooling, an oily resinous body deposits which becomes semi-crystalline. Ammonium carbonate is added, then solution of ammonia which produces a white precipitate. This is extracted with ether and the pure cocaine crystallises out from the ethereal solution. The acid used may be recovered by concentrating the mother liquors and precipitating with hydrochloric acid.

Another method is to dissolve crude cocaine in boiling water containing acetic acid. On cooling, precipitate with ammonium carbonate yielding a resinous yellow precipitate lighter than water. The solution is filtered and ammonia added. The cocaine is crystallised from ether.

The ecgonine contained in the resinous precipitate can be worked up. The residue is purified by crystallisation from alcohol and pure ecgonine precipitated by sodium carbonate. It is dissolved in methyl alcohol and the solution treated with anhydrous hydrochloric acid. Of the methylecgonine obtained, 20 g. is heated on a water-bath with benzoyl chloride, 20 g., until no more hydrochloric acid is evolved. The solution is added to cold water. Benzoic acid is precipitated. This is filtered out and the filtrate concentrated. The synthetic cocaine (termed coca-ethylene in the German Patent 47,713) is then precipitated from the filtrate by means of ammonia.—De Rosemont, *J. suisse Pharm.*, Apl. 29, 1920, per *Chem. & Drug.*, ii/1920, 934.

A tentative method for the determination of cocaine is described in *Methods of Analysis* (A.O.A.C., 1940). A portion of the mixed sample containing approximately 0.2 g. of alkaloid dissolved in 20 ml. of water with 2 drops of 10% hydrochloric acid is made alkaline with fresh saturated sodium bicarbonate solution and shaken out with petroleum ether. The petroleum ether is shaken with excess of N/50 sulphuric acid and the aqueous layer and washings from the petroleum ether back titrated with N/50 alkali using methyl red indicator. To the titration liquid 10 ml. of 2.5N sodium hydroxide is added and evaporated to about 10 ml. After cooling, the liquid acidified with 10% hydrochloric acid is extracted with chloroform, the chloroform evaporated spontaneously and the benzoic acid, after drying in a vacuum desiccator for 2 hours, is weighed.

Tests for Cocaine

PERMANGANATE TEST. When a drop of a solution of cocaine is placed on a dried film formed by a solution of potassium permanganate on a micro slide and examined under the microscope, oily drops are seen. If, however, the cocaine is dissolved in a saturated solution of alum, violet crystals of cocaine permanganate will quickly be observed. Alypin, tropacocaine and scopalamine produce crystals from aqueous solutions. Beta-eucaine, Stovaine, Novocain and holocain form no crystals with permanganate. Saporetti's bromine test distinguishes.

REICHARD'S TEST consists in adding a concentrated solution of sodium nitroprusside, drop by drop, to a cocaine salt solution containing at least 0.004 g. cocaine per millilitre. A precipitate of reddish crystals is formed which dissolves on warming and reappears after the liquid is cooled.

PISANI'S TEST. A wine-red colour is obtained by heating together cocaine or cocaine hydrochloride with a few drops of concentrated sulphuric acid containing 2% formamide. The colour soon disappears, giving place to a brownish-grey precipitate. The test is stated to detect 0.001 g. cocaine.—Autenreith, *Detection of Poisons and Powerful Drugs*.

With chromic acid and cobalt nitrate, Alypin behaves similarly to cocaine and eucaine and precipitates with usual alkaloidal reagents, caustic and carbonated fixed alkalis, and ammonia.

The four alkaloids, cocaine, truxilline, $C_{15}H_{21}NO_4$ (also called cocamine or isotropyl-cocaine), cinnamyl-cocaine and tropacocaine, $C_8H_{11}NO, C_6H_5CO$, are known to exist in coca leaves.

VITALI'S REACTION. Cocaine gives a reaction similar to atropine if the alkaloidal residue, after mixing with alcoholic solution of potassium hydroxide, is heated on the water-bath, but gives no reaction in the cold—the reaction in this case, however, is found to be due to isotropyl-cocaine as impurity.—*Yearb. Pharm.*, 1922, 7. Further experiments to show that the colour is due to esters of the tropic acid series.—*Yearb. Pharm.*, 1923, 24.

Cocainæ Hydrochloridum (B.P.). $C_{17}H_{21}O_4N, HCl = 339.6$. M.p. not lower than 197° , when placed in the heating bath at 193° . Specific rotation in 2% w/v aqueous solution, -70° to -72° . Ash limit, 0.1%. The substance of the *U.S.P. XII* has a specific rotation, in 2% aqueous solution, of -71° to -73° ; loss on drying over sulphuric acid, not more than 1%. Tests for acidity, carbonisable substances, cinnamyl cocaine and isotropyl cocaine are included.

It should not only be in good crystals, but should, by the following modification of MacLagan's Test, yield a distinctly crystalline precipitate of pure cocaine within 3 minutes when 1 grain is dissolved in 2 oz. of distilled water, and 6 to 8 drops of dilute solution of ammonia, *B.P.*, are added and well stirred. If more than 4% of amorphous alkaloid (principally truxilline) be present, there will be only a cloudiness. The precipitate redissolves after 24 hours or more, the cocaine being converted into methyl alcohol and benzoyllecgonine. Truxilline is highly toxic. (*Fr. Cx.* 1937 and *P.G. VI* give this test in modified forms.)

Cocæ Folia. The leaves from Bolivia, Peru and Ceylon contain cocaine as their chief alkaloid, whilst the Java leaf contains chiefly cinnamyl-cocaine, but little or no cocaine, chemical treatment being necessary to convert the alkaloids into cocaine. The genus *Erythroxylon*, which yields cocaine alkaloids and is indigenous to 4 continents, comprises about 80 species of which only 4 or 5 have so far been completely examined as regards alkaloidal content. The commercial supply comes from at least three distinct species.—*Plant Alkaloids*, T. A. Henry.

League of Nations Assay. The Health Committee have published the Committee of Experts' method for the assay of raw cocaine and coca leaves.—*Bull. Hlth Org. L. o. N.*, 1938, 429.

Suggested modifications to the method of the Committee of the Health Organisation of the League of Nations (*Quart. J. Pharm.*, 1938, 765) consist of firstly powdering the leaves finely rather than coarsely and, secondly, extracting the alkaloids from the acid by using 5 g. of sodium bicarbonate or 5.5 ml. N/1 sodium carbonate and three 30 ml. portions of ether.—A. W. K. De Jong, *per Quart. J. Pharm.*, 1939, 116.

Liquid Extract of Coca. The *B.P.C.* assay requires modification. The extraction of the alkaloids from ammoniacal solution should be made with successive portions of ether until complete, and sufficient dilute acid should be used to extract the alkaloids completely. The final ethereal solution must be washed with a little water to remove any traces of ammonium salts and the final residue dehydrated with absolute alcohol and heated at 80° for 2 hours to remove volatile bases.—W. A. Markwell, *Pharm. J.*, i/1935, 416.

Assay of Preparations containing Cocaine or Coca Alkaloids. Cocaine is not precipitated by sodium bicarbonate from solutions of its salts but after the addition of bicarbonate it can be extracted by immiscible solvents. Other alkaloids and local anaesthetics are only extracted very slowly under these conditions and if the solvent used is light petroleum, few are extracted. In the few cases where traces of alkaloids other than cocaine are extracted, they may be destroyed by treatment with potassium permanganate which does not affect cocaine appreciably in acid solution. The extracted residue or the original sample is dissolved in N/10 sulphuric acid and a 3% solution of potassium

permanganate in N/2 sulphuric acid is added until the colour indicates an excess. The solution is decolorised with oxalic acid, made alkaline with sodium bicarbonate and extracted with light petroleum. If permanganate treatment has not been employed, the other bases may be obtained by liberation with ammonia and extracting with a solvent.

Coca preparations contain cocaine and other ecgonine derivatives of similar constitution to cocaine, and also physiologically inert bases, such as the hygrines. Only cocaine and the related alkaloids come within the Dangerous Drugs Acts. In order to determine only the cocaine alkaloids, the bases should be liberated by sodium bicarbonate and a mixture of equal parts of ether and light petroleum used for the extraction. By this procedure the hygrines are not extracted. In the B.P.C. assay for Ext. Cocæ Liq., cocaine alkaloids instead of total alkaloids can be determined by adding 1 g. of sodium bicarbonate to the mixed sulphuric acid extracts, and shaking out first with 20 ml. of a mixture of equal parts of ether and light petroleum, and then with two successive portions, each of 15 ml., of the same mixture. The combined extracts are filtered, the solvent evaporated and the residual cocaine alkaloids dissolved in excess of acid and back titrated.—J. R. Nicholls, *Analyst*, 1936, 155.

The total alkaloids in coca leaves vary from about 0.5 to 1.5%, the cocaine alkaloids usually amounting to about 70 to 80%. A standard of 0.35 to 0.45% of cocaine alkaloids, calculated as cocaine, has been suggested for the B.P.C. liquid extract.

Determination of Cocaine in presence of Procaine Hydrochloride. Dissolve in water, treat with slight excess of sodium nitrate in presence of dilute sulphuric acid for three minutes. Add 10% sodium hydroxide and 1% β -naphthol solution, and compare the colour with that obtained from a solution of pure procaine hydrochloride. The amount of cocaine present is found by difference.—S. N. Chakravarti and M. B. Ray, per *Chem. Abstr.*, 1938, 1866.

Tabellæ Cocainæ Hydrochloridi (N.F. VII). Contain 91 to 109% of the labelled amount of cocaine hydrochloride, including all tolerances. Assayed by dissolving in water, making alkaline with ammonia, extracting with ether, reducing the ethereal extracts to half their volume, extracting with N/20 sulphuric acid and titrating the excess acid with N/50 sodium hydroxide, using methyl red indicator.

COCAINE SUBSTITUTES

Amydricainæ Hydrochloridum (B.P.C.). $C_{16}H_{26}O_2N_2.HCl$ = 314.7. M.p., about 169°. Loss at 100°, not more than 2%. Ash, not more than 0.1%.

Amylocainæ Hydrochloridum (B.P.). $C_{14}H_{21}O_3N.HCl$ = 271.6. M.p., 177° to 179°. Ash, not more than 0.1%.

Benzaminæ Hydrochloridum (B.P.C.). $C_{15}H_{21}O_2N.HCl$ = 283.6. M.p., about 268°, with decomposition. Loss at 100°, not more than 1%. Ash, not more than 0.1%. Eucainæ Hydrochloridum, U.S.P. XII, after drying at 100°, contains not less than 99% of $C_{15}H_{21}O_2N.HCl$; determined by titration in alcohol with N/10 sodium hydroxide using phenolphthalein indicator.

Benzaminæ Lactas (B.P.C.). $C_{15}H_{21}O_2N.C_3H_6O_3$ = 337.2. M.p., 152° to 156°. Loss at 100°, not more than 1%. Ash, not more than 0.1%.

Benzocaina (B.P.). $C_9H_{11}O_2N$ = 165.1. M.p., 90° to 91°. Ash, not more than 0.1%. *Æthylis Aminobenzoas*, U.S.P. XII, melts between 88° and 90°.

Butacainæ Sulphas (U.S.P. XII). $(C_{18}H_{30}N_2O_3)_2.H_2SO_4$ = 710.9. M.p., 100° to 103°; ash, not more than 0.2%. Complies with a test for carbonisable substances. (Butacaine sulphate is described in Vol. I under the trade name, Butyn.)

Butylis Aminobenzoas (*U.S.P. XII*). $C_{11}H_{15}O_2N=193.2$. M.p., 57° to 59° . Ash, not more than 0.15%.

Orthocaina (*B.P.*). $C_8H_9O_3N=167.1$. M.p., 141° to 143° . Loses not more than 1% at 100° . Ash, not more than 0.1%.

Phenacainæ Hydrochloridum (*U.S.P. XII*). $C_{18}H_{22}O_2N_2 \cdot HCl, 2H_2O=352.7$. Ash, not more than 0.1%; loss at 105° , not more than 7%; the dried substance on extraction with chloroform from ammoniacal solution, yields 87.5 to 90.5% of the base dried at 105° . M.p. of substance dried at 105° , not below 190° .

Procainæ Hydrochloridum (*B.P.*). $C_{12}H_{20}O_2N_2 \cdot HCl=272.6$. M.p., 154° to 156° . Ash limit, 0.1%. The *U.S.P. XII* substance leaves a negligible ash from 0.5 g. Procainum, m.p. 60° to 62° , Procainum hydrochloricum, m.p. 153° to 154° , and Procainum nitricum, m.p. 100° to 102° , are official in the *P. Helv. V*. The *Fr. Cx.* 1937 requires Chlorhydrate de *p*-Aminobenzoyl-diéthylaminoéthanol to have a m.p. of 154° to 155° .

Official quantitative methods for the determination of mixtures of procaine with any *p*-aminobenzoic acid due to decomposition of procaine, and of undecomposed procaine, and a tentative method for the determination of procaine in the presence of chlorbutol, cocaine, codeine, heroin, lactose and morphine are described in *Methods of Analysis* (*A.O.A.C.*, 1940, 590).

Tabellæ Procainæ Hydrochloridi (*N.F. VII*). Contain 92.5 to 107.5% of the labelled amount of procaine hydrochloride, including all tolerances. Assayed by extracting an ammoniacal solution with chloroform, filtering the chloroform extracts, removing the chloroform, dissolving the residue in N/10 sulphuric acid and back titrating with N/50 sodium hydroxide, using methyl red indicator.

A method for the determination of procaine in hypodermic tablets and injections, depending upon a diazo reaction followed by coupling with 1-amino 8-naphthol 3:6 disulphonic acid, is described. Tables are given correlating the value of the red compound in the colour produced with the amount of procaine present.—N. L. Allport and N. R. Jones, *Quart. J. Pharm.*, 1942, 243.

Tetracainæ Hydrochloridum (*U.S.P. XII*). $C_{15}H_{24}N_2O_2 \cdot HCl=300.8$. Loses not more than 1% when dried over sulphuric acid for 18 hours and then contains 86.5 to 88.5% of $(C_{15}H_{24}N_2O_2)$. M.p., 147° to 150° .

COLCHICUM

Colchici Cormus (*B.P.*). Contains not more than 2% of other organic matter. The corm dried at 65° contains not less than 0.25% of colchicine. Assayed by the same process as the seed, but subtracting the amount of the final residue which is insoluble in cold water. Colchici Cormus, *N.F. VII*, should yield not less than 0.35% of colchicine, and not more than 0.5% of acid-insoluble ash. Indian colchicum corm has a lower and more variable alkaloidal content; it is therefore a poor substitute for the British drug and there are difficulties in preparing standardised galenicals from it.

Assay of colchicum by phosphotungstic acid.—E. C. Davies, *Pharm. J.*, i/1921, 505. Iodine may also be employed as precipitant.

Estimation by shaking out the alkaloid with chloroform and precipitating with phosphotungstic acid (Scheibler's reagent). Precautions necessary in estimating colchicine due to the presence of an amino-acid grouping.—J. Grier, *Pharm. J.*, ii/1923, 87.

For the methods upon which the *B.P.* processes were formulated see Self and Corfield, *Quart. J. Pharm.*, 1932, 347.

Colchici Semen (*B.P.*). Other organic matter, not more than 2%. Contains not less than 0.3% of colchicine. Ash limit, 3%. Assayed by continuous extraction with alcohol, filtering the cooled and settled liquid, and evaporating to dryness; the solution of the residue in 20% *w/v* sodium sulphate solution is cleaned with ether, cleared with talc and filtered; an aliquot part of the filtrate is again cleaned with ether, shaken with chloroform, N/1 sodium hydroxide added and the alkaloid completely extracted with chloroform; the chloroform is evaporated, two portions of alcohol added and evaporated, and the residue dried at 100° and weighed. Colchici Semen, *U.S.P. XII*, yields not less than 0.45% of colchicine, when extracted by a lead subacetate process, the weight of residue insoluble in N/20 sulphuric acid with chloroform being deducted from the weight of residue from chloroform extraction. Colchique, *Fr. Cx.* 1937, by digestion with water, treatment with lead subacetate, followed by sodium phosphate and addition of sodium chloride before extraction with chloroform, finally drying at 105°, contains not less than 0.40% of total alkaloids. Semen Colchici, *P. Helv. V*, contains not less than 0.5% of colchicine and the powder for administration is adjusted with lactose to contain 0.4% of colchicine.

Extractum Colchici Liquidum (*B.P.*). Assayed after evaporation, by the process for Colchici Semen, it contains 0.27 to 0.33% *w/v* of colchicine.

Extractum Colchici Siccum (*B.P.*). Contains 0.9 to 1.1% of colchicine, assayed by the process for Colchici Cormus.

Tinctura Colchici (*B.P.*). Assayed similarly, contains 0.027 to 0.033% *w/v* of colchicine. Tinctura Colchici Seminis, *U.S.P. XII*, contains 0.036 to 0.044% *w/v* of colchicine.

Colchicina (*B.P.C.*). Ash, not more than 0.1%. Tests for limit of chloroform compound and of colchicine are described. Colchicinum, *P.G. VI*, $C_{22}H_{25}O_6N, \frac{1}{2}CHCl_3$, contains 87% of colchicine. Colchicinum, *P. Helv. V*, is the crystalline alkaloid, $C_{22}H_{25}O_6N + 1\frac{1}{2}H_2O$, containing from 6 to 7% of water of crystallisation.

Tabellæ Colchicinae (*U.S.P. XII*). Contain 90 to 110% of the labelled amount of $C_{22}H_{25}NO_6$, including all tolerances. In the assay, a weighed quantity of powdered tablets is first macerated for 5 minutes with light petroleum. The light petroleum is decanted through a filter and discarded, and the residue is then extracted twice with hot alcohol by gentle refluxing for 10 minutes. Each alcohol solution is passed through the filter which is then washed with hot alcohol until the runnings are colourless. The alcoholic extracts are then evaporated to dryness and the residue is dissolved in chloroform. The chloroform solution is filtered into a tared flask and the filter washed with chloroform until the runnings are colourless. After removal of the chloroform the residue is evaporated twice with 2 ml. of alcohol and finally dried at 100°.

COLOCYNTHIS

Colocynthis (*B.P.*). Should contain not more than 5% of seeds, 2% of outer sclerenchymatous pericarp, and should yield not more than 8% of acid-insoluble ash, and not more than 3% of light petroleum extractive dried at 100°. The *N.F. VII* limit of petroleum ether extractive is 2% dried over sulphuric acid for about 18 hours, and of acid-insoluble ash, 4%.

COLOPHONIUM

Colophonium (*B.P.*). Acid value, 150 to 180. Ash, not more than 0.1%. Resina, *U.S.P. XII*, should have a sp. gr. at 25° of 1.07 to 1.09, an acid number not less than 150, and should leave not more than 0.1% of ash; about 1 g. is used in determining the acid value.

Guaiaci Resina (*B.P.C.*). Should leave not more than 10% of matter insoluble in alcohol and not more than 4% of ash; should comply with a test for absence of colophony. Guaiacum, *N.F. VII*, gives no reaction for rosin with copper acetate; alcohol-insoluble residue, dried at 110° for 30 minutes, not more than 15%; acid-insoluble ash, not more than 2%. Résine de Gaïac, *Fr. Cx.* 1937, has an acid value of 89 to 97 and α_D about -17° .

COPAIBA

Copaiba (*B.P.*). Residue, when heated on a water-bath, 50 to 65%. Sp. gr., 0.960 to 0.995. Acid value, calculated on the non-volatile residue, 120 to 160. Optical rotation of the distilled oil, -7° to -35° . Copaiba, *N.F. VII*, has sp. gr. 0.930 to 0.995 at 25° and an acid value of the original balsam of 28 to 95; the residue insoluble in absolute alcohol and dried at 80° should be not more than 5%; the volatile oil, obtained by steam distillation, should not boil below 250° and in a 10 decimetre tube should show an angular rotation of not less than -7° at 25°.

Oleum Copaibæ (*B.P.C.*). Sp. gr., 0.895 to 0.908. Optical rotation, -7° to -35° . Refractive index at 20°, 1.495 to 1.500.

Terebinthina Canadensis. The balsam obtained from *Abies balsamea* Mill (*Pinaceæ*), known as Canada balsam. It has a refractive index approximating that of microscopic glass, and "sets" in a non-crystalline transparent condition, hence is used as a mounting medium. In preparing for use it has to be gently heated in an open dish for a week or more until a small quantity removed becomes brittle when placed on a cold slab. Canada balsam 1 part by weight in xylol, in turpentine, in benzol, and in chloroform, each 1 by measure, are prepared for microscopic use. The first mentioned is chiefly employed and is frequently designated "Xylol-Balsam."

CORIANDRUM

Coriandrum (*B.P.*). Limits: for foreign organic matter, 2%; for ash, 7%; and for acid-insoluble ash, 1%. Coriandrum, *N.F. VII*, should contain not more than 5% of foreign organic

matter, not more than 1.5% of acid-insoluble ash, and not less than 0.25% *v/w* of volatile oil.

The Food and Drug Administration of the U.S. Dept. of Agriculture defined coriander seed as the dried fruit of *Coriandrum sativum* L. Required to contain not more than 7% total ash, and not more than 1.5% of ash insoluble in hydrochloric acid.—*S.R.A., F.D., No. 2, Rev. 5, Nov. 1936.*

Oleum Coriandri (B.P.). Sp. gr., 0.870 to 0.884. α_D , +8° to +15°. $n_{D_{20}^{\circ}}$, 1.462 to 1.472. Should be soluble in 3 volumes of alcohol (70%, sp. gr. 0.8896 to 0.8901). The *U.S.P. XII* requires the oil to comply with the same solubility test; sp. gr. at 25°, 0.863 to 0.875; α_D , +8° to +15° at 25°; $n_{D_{20}^{\circ}}$, 1.4620 to 1.4720.

CREOSOTUM

Creosotum (B.P.). Sp. gr., not less than 1.070. Distillation commences at about 200°, and not less than 95% *v/v* is collected between 200° and 230°. Creosotum, *N.F. VII*, has a sp. gr. of not less than 1.076 at 25°. Not less than 90% *v/v* should distil between 203° and 220°.

Genuine beechwood creosote yielded 39% of monophenols, 26.48% of guaiacol, and 32.14% of creosol, $C_6H_3 \cdot CH_2 \cdot OCH_3 \cdot OH$, and homologues; pinewood creosote about the same but 20.3% guaiacol and 37.5% of creosol and homologues—all boiling between 200° and 210°.

Creosote is usually optically inactive but may be slightly dextrorotatory.

Creosoti Carbonas (B.P.C.). Sp. gr., 1.150 to 1.180. Ash, not more than 0.1%. Free creosote is excluded by the absence of any green colour developing on addition of ferric chloride to the alcoholic solution. The *N.F. VII* specifies a sp. gr. at 25° of not below 1.145; 85% of the separated creosote should distil between 200° and 220°, which should not respond to the tests for hydrocarbons and coal-tar creosote for Creosotum, *N.F. VII*. Creosotum carbonicum, *P. Helv. V*, yields not less than 90% *v/w* of creosote, which distils (apart from 2.83% which may pass over between 190° and 200°) between 200° and 220°, when determined by the following process:—Dissolve 20 g. in 60 g. of alcoholic potash and evaporate on a water-bath to 26 g. to 28 g. Add, drop by drop, dilute hydrochloric acid until distinctly blue to Congo red paper, and rinse into a graduated cylinder with 40 ml. water. After separation, the volume should be not less than 18 ml. Add 50 ml. of ether, transfer to a separator, draw off the salt solution and dry the ether with a little fused calcium chloride and transfer to a distillation flask. The distillate should comply with tests specified for Creosotum, *P. Helv. V*.

Calcii Creosotas (N.F. VII). Dried over sulphuric acid, it leaves on ignition 40 to 50% of CaO .

Guaiacol (B.P.). The liquid form has a sp. gr. of 1.116 to 1.125, and the crystals a m.p. of about 28°. Not less than 95% should distil between 200° and 210°. Guaiacol, *N.F. VII*, should have a sp. gr. at 25° of not below 1.112 for the liquid and of about 1.132 for the melted solid; the solid boils between 204°

and 206° and not less than 85% of the liquid distils between 200° and 210°.

A tentative method for the determination of guaiacol, guaiacol carbonate or potassium guaiacolsulphonate is described in *Methods of Analysis* (A.O.A.C., 1940, 608).

Guaiacolis Carbonas (B.P.C.). $C_{15}H_{14}O_5 = 274.1$. M.p., 85° to 88°. Ash, not more than 0.1%. Should comply with a test for absence of free guaiacol. The *N.F. VII* substance should leave not more than 0.1% of ash.

Potassii Guaiacolsulphonas (B.P.C.). $C_7H_7O_5SK = 242.2$. Should give not less than 35.2% of sulphated ash, corresponding to not less than 98% of the pure substance. The composition is incorrectly given as $C_6H_5(OCH_3)(OH)SO_3K$ (1 : 2 : 3). Potassii Guaiacolsulfonates, *N.F. VII*, on drying over sulphuric acid for 24 hours loses not more than 2%, and contains 95% of $C_7H_7O_5SO_3K$; assayed by digestion and evaporation with nitric acid and precipitation as barium sulphate.

Guaiacolsulfonate de Potassium, *Fr. Cx.* 1937, loses not more than 3% at 100°, contains not more than 2.42% of alkali as K_2CO_3 , and the dried salt contains 99% of the pure substance. Kalium sulfoguaiacolicum, *P.G. VI*, contains not less than 96.9% of $C_6H_5(OH)(OCH_3)SO_3K$ (1 : 2 : 4 and 1 : 2 : 5). Kalium guaiacol sulfonicum, *P. Helv. V*, is described as a mixture of salts of 1-oxy-2-methoxybenzene-4-(and 5)-sulphonic acids and is required to give from 35 to 39% of sulphated ash.

The potassium salts of guaiacolsulphonic acid may be determined by titration with N/10 sodium hydroxide to Poirrier-blue, followed by titration with N/10 hydrochloric acid using alizarin-yellow R as indicator.—*Brit. chem. Abstr.* (B), 1933, 171.

CRESOL

Cresol (B.P.). Sp. gr., 1.035 to 1.050. Distillation range, not more than 2% *v/v* below 188° and not less than 80% *v/v* between 195° and 205°. Not less than 90% *v/v* of cresol, *U.S.P. XII*, should distil between 195° and 205°. Sp. gr., 1.030 to 1.038 at 25°. Cresolum crudum, *P.G. VI*, and Cresolum crudum, *P. Helv. V*, contain not less than 50% of *m*-cresol when determined by nitration and weighing the trinitro-*m*-cresols produced. 10 g. of cresol is heated in a litre flask on a well-boiling water-bath for 1 hour with 30 g. of sulphuric acid; after cooling, 90 ml. of nitric acid is added all at once and when reaction has ceased, after standing 15 minutes, the contents of the flask are added to 40 ml. of water in a basin and the flask rinsed with a further 40 ml. of water. After cooling and standing for 2 hours the crystals of trinitro-metacresol are broken up with a pestle, transferred to a dried filter and washed with 100 ml. of water in small portions, dried for 1 hour at 50° and then for 2 hours at 100° (*P.G. VI*) or 95° to 98° (*P. Helv. V*). 1 g. metacresol = 1.74 g. of nitro-compound.

The method in *P. Helv. V* for the determination of *m*-cresol is inaccurate, particularly with samples containing more than 90% of *m*-cresol, owing to irregular nitration. An improved and accurate method is described.—H. Muhlemann, *Pharm. Acta Helvet.*, 1940, 15, 141.

British Standard Specifications:—

B.S.S. No. 522—1933, includes specifications for: (1) Orthocresol (522A), crystals or masses having a crystallising-point of 30.3° to 31.0° and a boiling-point of about 191°; (2) Metacresol (522B), colourless to very pale straw-coloured liquid or solid of sp. gr. 1.037 to 1.040 and having a crystallising-point of not below 10.5° and a boiling-point of about 202°; (3) Paracresol (522C), crystals or masses having a crystallising-point not lower than 34° and a boiling-point of about 202°.

B.S.S. No. 517—1933, describes Cresylic Acid of high orthocresol content. It must contain not less than 45% of orthocresol; the sp. gr. is from 1.045 to 1.050 at 15.5°, and 90% distils between 192° and 200°. The proportion of orthocresol is determined by observing the crystallising-point of a mixture of the sample and pure cineole under carefully controlled conditions.

B.S.S. No. 521—1933, describes the variety of commercial cresol known as Cresylic Acid, (50/55% Metacresol), a liquid having a specific gravity of 1.035 to 1.040 at 15.5° and of which approximately 92% distils between 199° and 204°. The proportion of metacresol is determined by a nitration process and weighing the washed solid trinitrometacresol produced.

B.S.S. No. 524—1933, includes specifications for Refined Cresylic Acids. Refined Cresylic Acid, Grade A, is controlled for colour, water content, specific gravity (1.035 to 1.050), distillation (not more than 5% below 192° and at least 90% below 205°), neutral oils and pyridine bases, sulphuretted hydrogen, and freedom from acids and alkalis. Refined Cresylic Acid, Grade B, may contain twice as much water (1%), 10 times as much neutral oils and pyridine bases (1% of each), and at least 80% must distil below 205°.

Tests to distinguish Carbolic Acid, Cresols and other Phenols.

TEST I. Dissolve 1 drop (0.05 g.) of the phenol in 10 ml. of strong hydrochloric acid in a mortar and add 0.5 g. of a mixture of NaNO_2 (1 part), NaNO_3 (1 part) and exsiccated Na_2SO_4 (2 parts). Stir well and allow to stand for 2 to 5 minutes. Note colour and pour 1 ml. of the acid mixture into excess of 10% aqueous NH_4OH and note change of colour.

Carbolic Acid gives a rich crimson colour in 2 minutes. On pouring the mixture into NH_4OH a deep emerald green results. If at the crimson stage 1 or 2 drops of 38% formaldehyde solution are added and the mixture stirred, the colour changes to rich purple and if now poured into NH_4OH a deep blue colour occurs. *Ortho-Cresol*. A dichroic solution is given in the acid, green being predominant. If a drop or two of formaldehyde solution is then added the green changes to blue (purple by transmitted light). If now poured into NH_4OH , only olive-green results. *Meta-Cresol*, *Para-Cresol* and *Cresol* give no distinctive results and are thus easily distinguished from carbolic acid and ortho-cresol. The presence of *p*-cresol also inhibits the reaction with carbolic acid or *o*-cresol, and the test cannot be used for detection of either of these in cresol. *B.P. Beta-Naphthol* and *Alpha-Naphthol* give crimson-purple and violet-purple respectively after standing 3 to 5 minutes in the acid mixture. The colours are destroyed by ammonia solution. The acid solution withstands heat and subsequent dilution with retention of colour better than with other phenols. *Thymol*, after stirring and leaving for 5 minutes, gives a pronounced green, becoming yellow on pouring into ammonia solution.

TEST II. Dissolve 1 drop of the phenol in 5 ml. to 10 ml. of strong HCl with a minute crystal (size of pin's head) of NaNO_2 . Slowly heat the mixture nearly to boiling; cool or dilute and pour into excess of dilute NH_4OH solution. Note the colour changes.

Carbolic Acid, *ortho-cresol* and *meta-cresol*: On pouring the acid solution into NH_4OH and warming, a deep blue colour results. *Para-cresol* gives no colour. In aqueous solution, acidified with acetic acid, together with a little NaNO_2 , and a few drops 1 to 2% CuSO_4 solution, a rich wine-red solution is given, turning to pink on dilution with water. *Guaiacol* gives a green with NH_4OH . *Resorcinol* gives successively brown, red, purple, violet and blue; on diluting with water, green, and on pouring the acid mixture into ammonia solution, a dichroic solution with brilliant red fluorescence, wine-purple to transmitted light. *Orcinol*, *phloroglucinol* and the *catechins* give rather poor reds or purples both before and after treatment with ammonia, *catechol* gives a distinct bluish-green in the acid mixture, and *pyrogallol* a purplish colour if heated with a nitrate in the acid mixture.—A. H. Ware, *Analyst*, 1927, 335.

The determination of meta- and ortho-cresols in mixtures of cresols can be made by formation of the aldehyde resins; the amount of ortho-compound can be found by the cineole freezing-point method and, by subtraction, the proportion of meta-cresol can be ascertained.—C. E. Sage and H. R. Fleck, *Analyst*, 1932, 567.

Liquor Cresolis Saponatus (B.P. Add. I). Should contain 47 to 53% *v/v* of cresol. Assayed by acidifying, extracting with ether, evaporating the ether, and steam distilling the residual liquid at 170°; the distillate is saturated with salt, extracted with ether, warmed to 170° and the residue weighed, the cresol distilled and the weight of water, etc., and non-volatile residue subtracted; the distilled cresol should have the sp. gr. and b.p. of cresol. Miscible in all proportions, up to 10% *v/v*, with water. **Liquor Cresolis Saponatus, U.S.P. XII**, is assayed by distillation with kerosene and extraction of the distillate with sodium hydroxide, when the proportion of cresol, measured by the increase in volume of the sodium hydroxide solution used, should be from 46 to 52% *v/v*; the cresol separated from this solution should comply with the distillation standard for cresol. **Liquor Cresoli saponatus, P.G. VI**, contains about 50% of crude cresol and 25% of fatty acids from linseed oil.

Assay of Cresols in Lysol. The following method is more suitable and accurate than that of the B.P. when only normal quantities of lysol are available. It can be used for less concentrated preparations of cresols and the higher homologues of phenol and for other coal tar disinfectants in which the phenols are dissolved in, or emulsified with, solutions of soaps or resins.

Weigh 25 to 100 g. of the liquid, according to the amount available and the phenolic content of the preparation, into a conical flask and shake with 100 ml. of water; add 15 to 50 g. of solid barium hydroxide and heat for one hour under a reflux condenser by immersing the flask in boiling water, shaking well at frequent intervals. After cooling, pour off the aqueous liquid and filter, washing the residue, and finally the filter, with hot barium hydroxide solution. Acidify the filtrate in a separating funnel with hydrochloric acid, saturate the mixture with calcium chloride and extract the liberated phenols with successive small portions of ether. Transfer the ethereal solutions to a small flask, evaporate off the ether, dry the phenolic residue to constant weight by heating on a water-bath and finally over sulphuric acid. The weight obtained gives the quantity of crude phenols in the weight taken, and the percentage by volume may be calculated from the specific gravity of the crude phenols obtained and that of the original liquid. The nature of the phenols recovered may be ascertained by submitting the residue to fractional distillation and weighing separately portions of the distillate collected within the boiling-ranges of phenol, cresols and the higher homologues.

The following are references to other methods:—

100 g. of lysol treated with excess 2% sulphuric acid, and the fatty acids and cresols extracted with 50 and 20 ml. of ether. The ethereal layer is dried over sodium sulphate and distilled, and the phenols collected between 180° and 230°.—A. H. Dodd, *J. Soc. chem. Ind., Lond.*, 1924, 931.

Cresols in lysols approximately estimated by steam distillation of 60 g. to 70 g. after acidifying with 30 ml. to 35 ml. of dilute sulphuric acid. The weight of cresol in the sample is obtained by multiplying the volume of cresols in the distillate by 1.04 and adding 1/50 of the volume of the aqueous layer. Method gives trustworthy results.—C. J. Jordan and F. Southerden, *Analyst*, 1921, 375.

Data given of a large number of examinations of castor oil and linseed oil lysols.—*Pharm. J.*, i/1921, 479.

Accurate results by distillation can only be assured when the soap is known to be free from volatile fatty acids. It is best to dissolve the sample in hot water in a separator, add a piece of stick caustic soda and shake until dissolved. Add excess of brine and separate. Redissolve in hot water, add caustic soda and again salt out; repeat again. Acidify the united alkaline liquors and extract

with benzene. Extract this solution with a small quantity of caustic soda, acidify and read off in the usual way.—G. F. W. Martin, *Yearb. Pharm.*, 1922, 142.

Lysols vary appreciably in characters and properties but very little adjustment will be necessary to make them comply with the official strength. The assay process is unnecessarily complicated and there is confusion between crude cresol and separated cresol. A large proportion of the higher phenols (non-volatile residue) is lost and it is useless to examine the separated cresol to see that it comes within the B.P. boiling-range.—*Pharm. J.*, ii/1932, 107.

The lysol supplied by the large manufacturers would comply with a requirement of miscibility with water up to 10%, or even 20%, of lysol, but does not, in the majority of cases, comply with the requirement of miscibility in all proportions. The statement of miscibility with ether does not hold for commercial samples.—G. R. Page, *Quart. J. Pharm.*, 1934, 369.

Chlorocresol (B.P. Add. III). $C_7H_7OCl = 142.58$. M.p., 64° to 66° . Residue on volatilisation on a water-bath, not more than 0.1%.

Chloroxilenol (B.P. Add. VI). $C_8H_9OCl = 156.5$. M.p., 114° to 115.5° . Ash, not more than 0.1%.

The Bactericidal and Fungicidal Action of Homologous Chlorophenols. Tables are given showing the germicidal activity of chlorophenol derivatives on *E. paratyphosus*, *B. paratyphosus*, pathogenic cocci, and acid-fast bacteria, and the fungicidal action on various fungi.

All the tests were carried out at 37° and in all cases 0.5 ml. of culture was added to 5 ml. of the various dilutions of the antiseptic substances. The method of culturing and preparing the organisms for the tests followed closely that described by Ruehle and Brewer (U.S. Dept. of Agriculture, Circular No. 198, Dec. 1931) in the case of *Eberthella typhi*, and transfers from the medication tubes into fresh media were made after exposures of 5, 10, and 15 minutes respectively and the readings taken after 48 hours' incubation. The "phenol coefficients" were calculated in all cases from the results of the ten-minute exposures to compounds under discussion on the one hand and to phenol on the other. A phenol control test always accompanied the tests with these compounds, carried out on any given day. Most of the derivatives were found to have a very low toxicity to mice, the minimum lethal dose (subcutaneously) varying from 0.6 to over 10 mg. per gramme body weight.—E. Klarmann, V. A. Shternov and L. W. Gates, *J. Lab. clin. Med.*, 1934, 19, 835, and 1934, 20, 40.

THE BACTERICIDAL ACTION OF ORTHOALKYL DERIVATIVES OF *p*-CHLOROPHENOL UPON ORGANISMS OF THE TYPHOID COLON GROUP

Minimum Concentrations Effective in 10 Min. (I) and Phenol Coefficient (II)

| | <i>Eberthella Typhi</i> | | <i>Escherichia Coli</i> | |
|------------------------|-------------------------|--------|-------------------------|--------|
| | I | II | I | II |
| <i>p</i> -Chlorophenol | 1 : 650 | 4.3 | 1 : 600 | 4.6 |
| Alkyl Radical | | | | |
| Methyl .. | 1 : 2,000 | 12.5 | 1 : 2,000 | 14.3 |
| Ethyl .. | 1 : 4,000 | 28.6 | 1 : 3,500 | 26.9 |
| <i>n</i> -Propyl .. | 1 : 14,000 | 93.3 | 1 : 12,000 | 85.7 |
| <i>n</i> -Butyl .. | 1 : 22,500 | 141.0 | 1 : 16,000 | 114.0 |
| <i>n</i> -Amyl .. | 1 : 25,000 | 156.0 | 1 : 20,000 | 154.0 |
| <i>sec</i> -Amyl .. | 1 : 7,000 | 46.7 | 1 : 5,000 | 41.7 |
| <i>n</i> -Hexyl .. | (1 : 3,250) | (23.2) | (1 : 5,000) | (35.7) |
| Cyclohexyl .. | > 1 : 4,000 | < 26.7 | > 1 : 2,000 | < 14.3 |
| <i>n</i> -Heptyl .. | (1 : 3,000) | (20.0) | (1 : 2,000) | (14.3) |
| Phenol (control) .. | 1 : 140—150 | 1.0 | 1 : 140—150 | 1.0 |

THE GERMICIDAL ACTION OF HOMOLOGOUS DERIVATIVES OF *o*-CHLOROPHENOL
UPON ORGANISMS OF THE TYPHOID-COLON GROUP

Minimum Concentrations Effective in 10 Min. (I) and Phenol Coefficients (II).

| | <i>Eberthella Typhi</i> | | <i>Escherichia Coli</i> | |
|------------------------|-------------------------|-----|-------------------------|-----|
| | I | II | I | II |
| <i>o</i> -Chlorophenol | 1 : 350 | 2.5 | 1 : 300 | 2.3 |

p-Alkyl Derivatives

| | | | | |
|-----------------------|-------------|--------|-------------|--------|
| Methyl .. | 1 : 1,000 | 6.3 | 1 : 700 | 5.4 |
| Ethyl .. | 1 : 2,750 | 17.2 | 1 : 3,500 | 29.2 |
| <i>n</i> -Propyl .. | 1 : 6,000 | 40.0 | 1 : 4,000 | 33.3 |
| <i>n</i> -Butyl .. | 1 : 13,000 | 86.7 | 1 : 5,000 | 38.4 |
| <i>n</i> -Amyl .. | 1 : 12,000 | 80.0 | 1 : 2,500 | 19.2 |
| <i>tert.</i> -Amyl .. | 1 : 4,500 | 32.1 | (1 : 2,500) | (17.9) |
| <i>n</i> -Hexyl .. | (1 : 3,500) | (23.3) | — | — |
| <i>n</i> -Heptyl .. | (1 : 2,500) | (16.7) | — | — |

Aromatic Derivatives

| | | | | |
|---------------------|-------------|------|-------------|------|
| 4-Benzyl .. | 1 : 5,000 | 35.7 | 1 : 3,500 | 26.9 |
| 6-Benzyl .. | 1 : 3,750 | 25.0 | 1 : 3,000 | 23.1 |
| Phenol (control) .. | 1 : 140—150 | 1.0 | 1 : 140—150 | 1.0 |

The effect of change of concentration on the relative activity of phenol and *parachlorometacresol*. By determination of the concentration exponent for phenol and *parachlorometacresol* by methods involving counting and calculated from the times for 50% death of *B. coli* (Type I), it was found that the latter bactericide increases in activity much more rapidly than phenol when concentrations are increased by the same amount. The reverse is true on decrease of concentration.—E. R. Withell, *Quart. J. Pharm.*, 1942, 313.

Determination of *p*-Chloro-*m*-xylene in Antiseptic Solutions.

20 ml. of solution is made alkaline with 2 ml. of 50% sodium hydroxide, the alcohol distilled off and the oils extracted by shaking twice with light petroleum. The light petroleum extract is washed twice with water, and the combined aqueous liquids are treated with 20% calcium chloride solution and filtered to remove soap. The filtrate and washings are acidified with hydrochloric acid and extracted with ether. The soap contains some adsorbed *p*-chloro-*m*-xylene which may be removed by three extractions with boiling alcohol, diluting, filtering, evaporating off the alcohol under reduced pressure, acidifying and extracting with ether. The mixed ethereal liquids are evaporated, the residue dissolved in the minimum quantity of alkali and the phenol compound precipitated by a current of carbon dioxide and extracted with ether. The ethereal solution is evaporated and the residue dried and weighed. The *p*-chloro-*m*-xylene obtained usually melts at about 110°; its identity can be confirmed after recrystallisation from benzene. If the solution contains a high proportion of essential oils, the phenolic residue may contain some oil which may be removed by treatment with light petroleum and alkali.—R. P. Merritt and T. F. West, *Analyst*, 1938, 257.

The following method has yielded satisfactory results for solutions containing a variety of soaps and essential oils. *Method.*—20 ml. of the antiseptic solution are pipetted into a separator and acidified with 6N sulphuric acid. The oily layer is extracted three times with ether (25 ml.), the combined ethereal extracts are washed three times with water (10 ml.) and the water washed with 15 ml. ether. The ethereal solutions are combined and dried over sodium sulphate and, after removal of the solvent, the residue is boiled for 45 minutes on a hot plate

under reflux with 20 ml. of a 4% solution of naphthalene- β -sulphonic acid in methyl alcohol. The solution is transferred to a separator with twice its volume of N sodium hydroxide solution and 20 ml. of petroleum spirit, and the mixture shaken vigorously. The petroleum spirit is extracted three times with N sodium hydroxide (10 ml.), a little alcohol being used if necessary to break the emulsions formed. The combined alkaline extracts are acidified with 6N sulphuric acid extracted three times with 25 ml. of ether, the ethereal extract is washed with water in the usual way and dried over sodium sulphate, the solvent is removed, the residue dried in a vacuum desiccator and weighed. In some instances the result at this stage is high and accordingly, as a general method, it is preferable to purify the crude phenol by dissolving in the smallest quantity of sodium hydroxide solution, diluting with water to 60 ml., and precipitating with carbon dioxide. The suspended phenol is then extracted with ether, separated, dried in the usual manner and weighed. This process may not be successful if essential oils containing phenols are included in antiseptics, although this appears unlikely in view of the oils available.—D. McNicoll, R. P. Merritt, and T. F. West, *Analyst*, 1939, 261.

Pentachlorophenol

Pentachlorophenol is a weak acid, soluble in alcohol. It is stable and melts at 190-2°. The sodium salt is soluble in water. It is used in the field of industrial preservation being a very efficient fungicide against widely differing types of organisms. The sodium salt is equally toxic.—T. S. Carewell and H. K. Nason, *Industr. Engng Chem.*, 1938, 30, 622.

CROCUS

Crocus (B.P.C.). Should lose not more than 12.5% of moisture and then yield not less than 60% of alcohol (60%) extractive; styles and anthers, not more than 8%, and foreign organic matter, not more than 2%; ash, not more than 7.5%; light petroleum extractive, not more than 1%. The water extract of 0.02 g. in 100 ml. yields a colour similar to and not less intense than 0.1% *w/v* potassium dichromate solution. The *N.F. VII* allows not more than 10% of yellow styles, not more than 2% of foreign organic matter, not more than 7.5% of total ash, and not more than 1% of acid-insoluble ash; a similar test for intensity of colour is included.

The Food and Drug Administration of the U.S. Dept. of Agriculture defined saffron as the dried stigma of *Crocus sativus* L.; required to contain not more than 10% of yellow styles and other foreign matter, not more than 14% of volatile matter when dried at 100°, not more than 7.5% of total ash, and not more than 1% of ash insoluble in hydrochloric acid.—*S.R.A., F.D., No. 2, Rev. 5*, Nov. 1936.

Adulteration of saffron with carthamus flowers and calendula can be detected by the orange-red or clear-blue fluorescence of the material when exposed to ultra-violet light.—A. Castiglione, *Ann. Falsif.*, 1933, 26, 41.

CUPRUM

Cupri Sulphas (B.P.). $\text{CuSO}_4 \cdot 5\text{H}_2\text{O} = 249.7$. Estimated by interaction of the cupric salt with potassium iodide in solution containing 10% of acetic acid, the liberated iodine being titrated with standard thiosulphate solution, using starch mucilage as indicator. Should contain from 98.5% to the equivalent of 101% of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. No standard or method of assay was included in the *B.P. '14*. Cupri Sulphas, *U.S.P. XII*, contains from 63 to 66.8% of anhydrous compound, equivalent to not less than 98.5% of the crystallised substance.

Detection and Determination of Copper. Copper can be detected and determined by using sodium diethylthiocarbamate, $N(C_2H_5)_2CS_2Na$. The reagent is used as a 0.1% solution in water. On adding the reagent to 10 times its volume of test solution, previously made alkaline with ammonia, a golden-brown turbidity is produced in the presence of Cu. The determination is carried out by adding 10 ml. of reagent to the test solution containing 0.01 to 0.1 mg. of copper and made alkaline with ammonia. The resulting solution is diluted to 100 ml. and the colour of the liquid is compared with that of the liquid obtained by similarly treating solutions containing known amounts of copper. If preferred the coloured compound may be extracted from the aqueous solution by shaking with successive portions of carbon tetrachloride and the colour of the mixed extracts compared with that of standards treated similarly. Iron, if present, must be oxidised to the ferric condition, 2 g. of citric acid is then added and the liquid made more alkaline than pH 9 with ammonia. The copper is then determined as above, the coloured compound being extracted with carbon tetrachloride.

For a modified method in which acacia is used to stabilise the coloured compound, see *Analyst*, 1937, 657.

The coloured compound should be extracted with carbon tetrachloride before comparing the colours. The precipitation of ferric hydroxide causes a serious loss of copper and the diethylthiocarbamate reagent is too sensitive for medicinal substances.—N. Evers and L. A. Haddock, *Pharm. J.*, 1/1934, 452.

Small amounts of copper may be detected and determined by using benzoin oxime (*syn.* Cupron), $C_6H_5 \cdot CHOH \cdot C : NOH \cdot C_6H_5$. The reagent is used as a 1% solution in alcohol. (20 ml. of the solution to be tested is made slightly alkaline with ammonia and 1 ml. of the reagent added.) In the presence of copper a green precipitate is produced either at once or after some hours, according to the proportion present. In the absence of tartrate ions, precipitates are also given by Co, Ni, Pb, Fe and Al. For the determination of copper, the test solution, containing not more than 0.05 g. of copper, is treated with excess of solution of ammonia, raised to the boiling-point, and then treated with a slight excess (about 5 ml.) of a 4% alcoholic solution of the reagent. The precipitate is washed free from soluble salts with hot 1% ammonia solution, and then with hot alcohol to remove excess of the reagent. The precipitate is dried to constant weight at 110°. Each gramme of residue is equivalent to 0.2202 g. of Cu. In the presence of any interfering element, a solution of sodium potassium tartrate must be added to the test solution.

Minute amounts of Cu are found in chemicals and galenicals. In view of the value of such traces in hæmoglobin formation it is unlikely that they would be harmful.—N. Evers and L. Haddock, *Quart. J. Pharm.*, 1932, 458.

Copper in various chemicals ranged from 0.4 parts per million in hydrochloric acid to 68 in ferric glycerophosphate, and in galenicals from 4 in ammoniated tincture of valerian to 580 in *B.P.* 1914 extract of ergot.—N. Evers and L. A. Haddock, *Quart. J. Pharm.*, 1932, 458.

Detection and Determination of Copper and Nickel. Copper and nickel may be detected and determined by using salicylaldoxime, $C_6H_5 \cdot OH \cdot CH : NOH$. The reagent is used as a solution obtained by dissolving 1 g. in 5 ml. of alcohol and diluting to 100 ml. with water. The test solution is acidified with acetic acid and one-twentieth of its volume of the reagent solution is added. In the presence of copper an immediate precipitate is produced. **Determination of copper.**—The test solution, containing not more than 0.1 g. of copper, is treated with sodium hydroxide solution until a precipitate appears, and is then acidified with acetic acid. 50 ml. of the reagent solution is added and the liquid is stirred to coagulate the precipitate, and filtered. The precipitate is washed with cold water and dried to constant weight at 100° to 105°. Each gramme of precipitate is equivalent to 0.1894 g. of Cu. **Detection and determination of nickel.**—The test solution is adjusted to pH 7 to 8 and the reagent added. A green flocculent precipitate is produced in the presence of nickel. If traces only of nickel are present the solution becomes opalescent. The precipitate is stirred to effect coagulation, then filtered, washed, and dried at 100°. Each gramme of precipitate is equivalent to 0.1774 g. of Ni. Nickel and copper may be separately determined in mixtures by precipitating the copper first as described above in acid solution and then adjusting the filtrate and washings to pH 7 to 8 by means of ammonia, when the nickel complex is precipitated.

5-bromoanthranilic acid was shown to be superior to the unsubstituted acid

as an analytical reagent for a number of bivalent metals. *Reagent solution*.—5 g. of 5-bromoanthranilic acid were neutralised with sodium hydroxide and the solution diluted to 500 ml. The neutral solution gave no precipitates with calcium, barium, strontium and magnesium. *Determination of copper*.—A copper sulphate solution was diluted to 100 ml., a few drops of 5N acetic acid added, and the solution heated. 50 ml. of reagent were added per 0.05 g. of metal and gentle heating was continued for 30 minutes. Copper bromo-anthranilate was filtered off on a No. 4 sintered glass crucible, washed with hot water and dried at 105° to 110° for one hour. Factor for copper = 0.1288. In the presence of ammonium acetate and Rochelle salt, it was found necessary to use a threefold excess of reagent. *Determination of cobalt*.—The procedure was the same as for copper. Factor for cobalt = 0.1206. *Determination of nickel*.—Nickel bromoanthranilate was sparingly soluble in hot water, but satisfactory results were obtained by washing with a solution containing 0.1% of 5-bromoanthranilic acid. The precipitate was finally washed with a little cold water. Factor for nickel = 0.1200.—R. J. Sherman, *J. Soc. chem. Ind., Lond.*, 1942, 164.

Detection and Determination of Nickel. Nickel may be detected and determined by means of dimethylglyoxime (*syn.* diacetyldioxime), $\text{CH}_3\text{C}:(\text{NOH})\text{C}:(\text{NOH})\text{CH}_3$. The reagent is used as a 1% solution in alcohol. A few drops of the reagent are added to the boiling acidified test solution, and the mixture is then rendered alkaline with solution of ammonia. In the presence of nickel, red needles are produced. In the presence of a large excess of cobalt (for example, in testing for nickel in cobalt compounds), 2 g. of the cobalt compound is dissolved in 20 ml. of water, 40 ml. of 10% potassium cyanide solution is added, followed by 10 ml. of N/1 sodium hydroxide and 10 ml. of 20 vol. hydrogen peroxide. The mixture is heated in a water-bath for 10 minutes, 20 ml. of 10% ammonium chloride solution added and then 2 ml. of the reagent. The solution is then evaporated to dryness on a water-bath. In the presence of nickel red flocks separate during evaporation, or if the proportion is extremely small the residue, which is otherwise yellowish, will be tinged with pink. For the determination of nickel, the test solution should contain not more than 0.1 g. of Ni in 200 ml. After precipitation as above, the crystals are filtered off after 1 hour, washed with hot water and dried at 115° for 45 minutes. Each gramme of residue, $(\text{C}_4\text{H}_7\text{O}_2\text{N}_2)_2\text{Ni}$, is equivalent to 0.2032 g. of Ni. Nickel may also be determined colorimetrically. In the absence of cobalt and copper a slight excess of bromine water is added, followed by ammonia solution until the bromine colour is removed. A few drops of the reagent are then added. The intensity of the red colour produced is compared with that obtained with known amounts of nickel.

Insecticides. The specification of the Association of British Insecticide Manufacturers (Bulletin No. 82: Ministry of Agriculture and Fisheries) requires:

COPPER SULPHATE to contain not less than 98% crystallised copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$);

BORDEAUX POWDER to contain no water-soluble copper, and the equivalent amount of copper to be declared;

CHESHUNT COMPOUND to consist of 2 parts of copper sulphate and 11 of ammonium carbonate by weight, and to contain not less than the equivalent of 3.8% Cu;

BURGUNDY POWDER to contain not more than 2% alkalinity expressed as Na_2CO_3 and no water-soluble copper, the whole of the powder to pass through a 30-mesh B.S. sieve.

Organic Copper Compounds.

Cupri Alloxanas. $(\text{N}_2\text{C}_4\text{O}_4\text{H})_2\text{Cu}$. A bluish-green flocculent powder slightly soluble in water. The relative insolubility is a disadvantage from the therapeutic standpoint.

Cupri Glycinas. $(\text{NH}_2\text{CH}_2\text{COO})_2\text{Cu}$. A blue flocculent silky powder soluble about 1 in 200 of water at 15°, but more than twice as soluble at 40°. 0.06 g. per kilo in 0.5% solution intravenously killed a rabbit in 8 minutes, whilst a control of Salvarsan in proportion 0.1 g. per kilo was tolerated, hence the substance has a toxic dose of about 2 g. for an average man.

Cupri Hippuras. $(\text{C}_6\text{H}_5\text{CO}\cdot\text{NHCH}_2\text{COO})_2\text{Cu}$. A bluish-green crystalline powder. Almost insoluble in water, but soluble about 1 in 200 of a mixture of glycerin and water equal parts. It is more soluble in pure glycerin.

DERRIS

Derris (*B.P.C. Supp. V*). By continuous extraction with carbon tetrachloride, concentrating, and standing at 0° (seeding if necessary), filtering and air-drying not above 40°, crystals of the carbon tetrachloride compound equivalent to not less than 2% of rotenone are obtained; (factor 0.719). Ash, not more than 6%; acid-insoluble ash, not more than 2%. Derris *Præparata*, *B.P.C. Supp. V.*, contains 1.5 to 2.5% of rotenone.

Lonchocarpus (*B.P.C. Supp. V*). Determined by the method for Derris, contains not less than 2% of rotenone.

The value of derris root as an insecticide is now assessed upon a combination of rotenone and total ether-soluble extract contents.—G. A. Freak, *Chem. & Ind.*, 1942, 430.

Determination of Rotenone. The powdered material is mixed with 5% of decolorising charcoal and extracted by percolation with ethyl acetate. Percolation is carried out in percolators which slide inside other metal tubes which are permanently fixed in the water jacket. The drawn out ends of the percolators pass through rubber stoppers into filter flasks immersed in cold water. The diameter of the percolators is about $\frac{3}{4}$ in. for 10 to 20 g. of material, 1 in. for 20 to 40 g., and $1\frac{1}{2}$ in. for 40 to 100 g., each percolator being about 18 in. long. In the tapering ends of the two larger tubes, Gooch crucible discs are placed on which rest discs of filter-paper with a wad of cotton wool. The amount of material used for extraction should be sufficient to yield about 1 g. of rotenone. After extraction a weighed quantity of pure rotenone is added to the resins sufficient to bring the proportion present to at least 40%, and 1 g. is added if the amount present is already up to or above 40%, and the whole is warmed and mixed and then left overnight in a desiccator containing carbon tetrachloride. The crystals are broken up, washed with solution of rotenone in carbon tetrachloride until no further colour is obtained and dried at about 40° for 6 hours. The purity of the crude carbon tetrachloride complex varies between 91 and 96%; the mean value, 94%, may be used in approximate routine work. The purity can be accurately determined by triturating the complex with a saturated solution of rotenone in dehydrated alcohol, leaving the mixture overnight in a desiccator containing alcohol, filtering, washing with alcohol, drying at 100° and weighing.—R. R. le G. Worsley, *J. Soc. chem. Ind., Lond.*, 1936, 349T. See also *ibid.*, 1937, 13T, 175T.

Although there is usually good agreement, occasionally large differences occur between the results of carbon tetrachloride extraction and cold chloroform extraction, and the former cannot be relied on to extract the rotenone completely. The following process is based on that of Beach (*Soap*, 1936, 12, 109). 30 g. of powdered root is shaken for 2 hours with 300 ml. of chloroform, then left overnight and shaken for another hour. Alternatively, the mixture is shaken for a short time, left overnight, shaken by hand at intervals during the next day and then on the following day for a short time before continuing the assay. The liquid portion is poured off into a filter funnel large enough to hold sufficient to give 200 ml. of filtrate and the funnel is covered with a clock-glass. The filtrate is collected in a 200 ml. graduated flask, precautions being taken to avoid evaporation. The chloroform is removed from the filtrate by distillation until about 50 ml. remains; this is removed practically completely on the water-bath, the last traces being removed by heating on the water-bath under reduced pressure. 10 ml. of carbon tetrachloride is added and removed, first on a boiling water-bath and then at a lower temperature with a vacuum pump, the process being repeated with 5 ml. of carbon tetrachloride. The residue is dissolved in 15 to 25 ml. of carbon tetrachloride saturated at 0° with rotenone, the solution cooled, seeded if necessary with a crystal of rotenone-carbon tetrachloride complex and set aside in a cool place for 2 days, then in ice for 3 hours. The crystals are then filtered off and weighed. The error due to change in volume of the chloroform due to dissolution of the extracted matter is small and its effect is somewhat compensated by evaporation of the chloroform during filtration. The purity of the rotenone complex may be ascertained if required by determining the specific rotation of a 5% w/v solution in benzene. The pure complex gives a rotation of -16.25°

and it is sufficiently accurate to assume that the purity is proportional to the rotation. In the so-called Sumatra type of derris root there is no crystallisation of complex even on storing at 0°, and in these cases 2 g. of pure rotenone complex must be added to the extract from 40 g. of root.—W. M. Seaber, *J. Soc. chem. Ind., Lond.*, 1937, 168T.

The following method, recommended for inclusion in the B.P.C., is based upon the work of H. E. Coomber, J. T. Martin, and S. H. Harper (*J. Soc. chem. Ind., Lond.*, 1942, 111):—

Weigh accurately 20 g., in moderately fine powder, and extract for three hours by continuous extraction with boiling chloroform; remove most of the chloroform and transfer the residual solution to a 100 ml. conical flask, washing out the extraction flask with carbon tetrachloride, and evaporate almost to dryness on a water-bath in a current of air and, finally, for about five minutes under reduced pressure. Dissolve the extract in 15 ml. of hot carbon tetrachloride, evaporate off the solvent as before and repeat the solution and evaporation with a further 10 ml. of carbon tetrachloride. To the residue add 25 ml. of carbon tetrachloride saturated at 0° with pure rotenone and dissolve by heating gently under a reflux condenser, cool in ice for ten minutes, add one or two small crystals of rotenone-carbon tetrachloride compound if necessary and rotate the flask until crystallisation is apparently complete. If only a small quantity of crystalline matter separates dissolve 1 g., accurately weighed, of pure rotenone in the solution and repeat the crystallisation, deducting in the calculation of the result of the assay the weight of pure rotenone added. Place the flask in ice and allow it to stand at 0° overnight. Filter off the crystals in a suitable weighed crucible, wash the flask and crystals with 15 ml. of ice-cold carbon tetrachloride saturated with rotenone and dry the crucible with its contents at 40° for one hour. Add 25 ml. of dehydrated alcohol saturated at 0° with pure rotenone to the contents of the stoppered crucible, break up the crystalline mass by stirring and allow the closed crucible and contents to stand at 0° for four hours; remove the alcohol by suction, wash the residue of rotenone at 0° with 10 ml. of the dehydrated alcohol saturated with pure rotenone, dry at 105° and weigh. Remove the crystals, mix well, dissolve 1 g., accurately weighed, in sufficient benzene to produce 25 ml. at 20° and determine the optical rotation in a 10-cm. tube at 20°. Calculate the purity of the rotenone obtained by multiplying the observed rotation in degrees by 100 and dividing by 9.04, and calculate the percentage of pure rotenone in the sample after making allowance for the rotenone added during the process of the assay. *Note*:—The saturated solution of rotenone in carbon tetrachloride is prepared by dissolving 2.72 g. of pure rotenone in hot carbon tetrachloride, cooling, and making up to 1000 ml. at 0°. The saturated solution of rotenone in alcohol is prepared by dissolving 1.244 g. of pure rotenone in absolute alcohol and making up to 1000 ml. at 0°.

Commercial samples examined by the carbon tetrachloride process have been shown to contain from nil to 6.9% of rotenone.—*Bull. imp. Inst., Lond.*, 1933, 32, 469.

The carbon tetrachloride method of determining rotenone gives results which are low if the rotenone content of the resin (either isolated or present in the root) is below about 17%, and is seriously in error if the rotenone content of the resin is below 10%. It fails completely for resins of very low rotenone content. A resin may contain up to 15% of rotenone, i.e., 2.4% of rotenone in the root, and yet give no rotenone by the standard method of determination. The rotenone-carbon tetrachloride crystals which separate in the determination of rotenone are at most 90 to 95%, probably only 80 to 90%, pure.—R. S. Cahn and J. J. Boam, *J. Soc. chem. Ind., Lond.*, 1935, 37T.

Determination of rotenone in derris root.—Th. M. Meijer and D. R. Koolhaas, *Industr. Engng. Chem. (anal. Edn.)*, April, 1940, 205.

Approximate Colorimetric Determination. The violet colour produced by the action of strong sulphuric acid containing nitrite on derris extract is proportional to the ether extract. 1 g. of air-dried derris powder is shaken for five minutes with 10 ml. of acetone. The suspension is then filtered, and 1 ml. of the filtrate is diluted to 25 ml. with distilled water. 0.2 ml. of the milky solution is pipetted into a dry test-tube and treated with 5 ml. of a 10% solution of sodium nitrite in strong sulphuric acid, added slowly. The intensity of the colour is measured in a Pulfrich "Stufenfotometer" with a S53 (wave length 550) filter. The percentage of ether extract can be calculated from these measurements. An approximate determination without the use of a photometer may be

made by employing solutions of cobalt chloride in water and in alcohol as colour standards. These solutions are stable if kept in sealed tubes.—T. M. Meijer, per *Quart. J. Pharm.*, 1937, 86.

Direct insecticidal tests show that no single chemical method of assay truly assesses the relative potencies of derris. The toxicity is determined not only by the rotenone content but by the precursors of deguelin and toxicarol also.—*Rothensted Experimental Station Report for 1935*, p. 58.

Constituents. In addition to rotenone, a dimorphic substance (m.p. 189° and 192° to 194°), isomeric with tephrosin, $C_{13}H_{12}O_7$, and having insecticidal properties, has been isolated from derris. Published data on the relative toxicities of rotenone, deguelin, tephrosin, and toxicarol are inapplicable to the assessment of the toxicity of derris resin, as the last three compounds do not occur as such (or, at most, in very small amounts) in derris resins. The conclusion, often drawn from published data, that the value of a derris root or resin can only be assessed by its rotenone content, is quite unjustified.—R. S. Cahn and J. J. Boam, *J. Soc. chem. Ind., Lond.*, 1935, 42T.

The toxicity is due to rotenone, deguelin and an unidentified compound. The latter is crystalline and is levorotatory in benzene. When treated with alcoholic potash it gives a high yield of optically inactive toxicarol. It has one-tenth the insecticidal power of rotenone. **Stability.** Derris is stable for years in tins stored under dry, cool conditions. Exposure to light causes loss of activity.—J. T. Martin, *J. Soc. chem. Ind., Lond.*, 1937, 79T.

Rotenone

Colour Test. To 1 ml. of solution in acetone add 1 ml. of diluted nitric acid (1 part acid and 2 parts water) and allow to stand for 30 seconds. Dilute with 8 or 9 ml. of water and add 1 ml. of strong solution of ammonia. A blue colour is produced with 0.0001 g. of rotenone.—H. A. Jones and C. M. Smith, *Industr. Engng Chem. (anal. Edn.)*, 5, 1, 75.

The purity of commercial rotenone can be determined colorimetrically by means of the red colour produced when nitric acid and sodium nitrite are added to a mixture of a solution of the sample in acetone and an equal volume of 10% solution of potassium hydroxide in 95% alcohol. Standard conditions must be employed.—C. R. Cross and C. M. Smith, *J. Ass. off. agric. Chem., Wash.*, 1934, 17, 336.

Detection and Determination of Rotenone in Insecticides. 1 to 2 ml. of sample is diluted to 5 ml. with chloroform, 5 ml. of 10% solution of thymol in chloroform is added, followed by 3 ml. of a mixture of nitric acid 0.2 ml. and hydrochloric acid 100 ml. In the presence of rotenone a bluish-green to blue colour develops on shaking for about 30 seconds.

Determination. A. To 10 ml. of a chloroformic dilution of the insecticide containing 0.05 to 2.5 mg. of rotenone per ml. is added 10 ml. of thymol 10% in chloroform and 2 ml. of a mixture of 100 ml. of hydrochloric acid with 2.5 ml. of solution of hydrogen peroxide. After shaking for 1 minute the mixture is exposed to ultraviolet light. A greenish-blue coloration appears in 15 minutes and after 30 minutes the colour is compared with that produced by known quantities of rotenone similarly treated.

B. The insecticide is diluted with acetone instead of chloroform as in the above test, and the thymol solution is also prepared with acetone. 10 ml. of the thymol solution is added to the insecticide dilution, followed by 0.1 ml. of solution of hydrogen peroxide and 5 ml. of hydrochloric acid. The mixture is warmed to 20° by immersion in warm water and the colour produced after 20 minutes is compared with that obtained by similarly treating known amounts of rotenone. In the presence of kerosene or extracts of pyrethrum, similar additions should be made to the standards.—H. D. Rogers and J. A. Calamari, *Industr. Engng Chem. (anal. Edn.)*, 1936, 135.

A survey of the correlation of toxicity and optical activity of derris derivatives.—R. S. Cahn, *J. Soc. chem. Ind., Lond.*, 1936, 259T.

Toxic Constituents of derris root.—T. A. Buckley, *J. Soc. chem. Ind., Lond.*, 1936, 285T.

DEXTROSUM

Dextrosom (B.P.). $C_6H_{12}O_6=180.1$. Loses not more than 2.5% at 105°, and then a well-boiled 10% w/v solution has a specific rotation of not less than +52°. Residue after ignition and

re-ignition with sulphuric acid, not more than 0.1%. Dextrosum, U.S.P. XII, loses 8 to 10% at 100°. Ammonia solution is added to the solution for the α_D determination, which on the dried substance is +52.5° to +53.0° at 25°. Dextrose which does not conform to the official limits for moisture may be used for the preparation of aqueous solutions, provided it meets all other requirements and allowance is made for the different water content. The moisture limits of 8 to 10% are determined at 100°. Glycosum, P. Helv. V, is pure anhydrous dextrose containing not more than 1% of moisture.

The Food and Drug Administration of the U.S. Dept. of Agriculture defined dextrose as the product chiefly made by the hydrolysis of starch or a starch-containing substance, followed by processes of refining and crystallisation. *Anhydrous dextrose* was required to contain not less than 99.5% of dextrose and not more than 0.5% of moisture. *Hydrated dextrose* was required to contain not less than 90% of dextrose and not more than 10% of moisture including water of crystallisation. *Glucose*, mixing glucose, confectioner's glucose, was described as a thick, syrupy, colourless product made by incompletely hydrolysing starch, or a starch-containing substance, and decolorising and evaporating the product and was required to contain on a basis of 41° Baumé, not more than 1% of ash, chiefly chlorides and sulphates.—S.R.A., F.D., No. 2, Rev. 5, Nov. 1936.

Reducing Sugars.

Determination. The method is based on the reduction of an alkaline solution of potassium ferrocyanide and titration of the resulting ferricyanide with ceric sulphate, using *o*-phenanthroline ferrous sulphate as indicator. Approximately N/100 ceric sulphate is prepared by dissolving the required amount of ceric sulphate in 500 ml. of water to which 100 ml. of a 1 to 1 sulphuric acid solution has been added, and diluting to 1 litre. A 0.025M solution of the indicator is prepared by dissolving 0.695 g. of crystalline ferrous sulphate and 1.485 g. of *o*-phenanthroline monohydrate in water to 100 ml. The alkaline ferrocyanide solution contains 4 g. of potassium ferrocyanide and 24 g. of sodium carbonate per litre. The ceric sulphate is standardised against pure anhydrous dextrose. 10 ml. of a solution containing between 5 and 10 mg. of glucose is warmed on a steam-bath with 25 ml. of the alkaline ferrocyanide solution for exactly ten minutes, the solution is cooled to room temperature, 10 ml. of 5N sulphuric acid and 2 drops of the indicator are added, and the solution is titrated with the ceric sulphate solution. A correction has to be made for the volume of the ceric sulphate solution required by the indicator, which is obtained by a blank titration. If used for the determination of reducing sugars in a plant extract the latter must be clear and colourless. This is obtained by extracting the plant material with alcohol (80%), evaporating to a low bulk, adding a saturated solution of neutral lead acetate and removing the excess of lead by disodium phosphate; decolorising charcoal is added and, after allowing to stand for thirty minutes with occasional stirring, the mixture is filtered through a thin layer of talc on a filter paper. The extract is diluted to a definite volume so that 10 ml. of the dilution will not contain more than 10 mg. of reducing sugar. For the determination, 10 ml. of the dilution is treated as in the above standardisation of the reagent. For the determination of the sucrose 50 ml. of the plant extract is neutralised to the red colour of methyl red with acetic acid, 2 to 4 drops of a 1% solution of Wallerstein invertase scales are added and the solution allowed to stand for two hours at 28°. The solution is then diluted to the required volume and the reducing sugars in 10 ml. determined. As the invertase solution has a slight reducing action it is necessary to carry out a blank determination on this reagent.—W. Z. Hassid, *Industr. Engng Chem. (anal. Edn.)*, 1936, 138.

Reducing sugars may be determined quantitatively by titration of boiling alkaline solution with potassium ferricyanide containing methylene blue as indicator. The method has also been used for sucrose, lactose in milk, glucose in urine and mixtures of maltose and glucose.—S. W. Cole, *Biochem. J.*, 1933, 723.

The potentiometric estimation of glucose with potassium ferricyanide in sodium carbonate solution gives a greater accuracy as compared with Fehling method.—H. T. S. Britton and L. Phillips, *Analyst*, 1940, 149.

Fructose.

Iodometric Determination. The method is based upon the reduction of a solution of copper carbonate. After the reduction the precipitated cuprous oxide is removed by filtration and the cupric ions in the filtrate determined by the addition of sulphuric acid and potassium iodide and titration of the liberated iodine with sodium thiosulphate; from a blank titration of the volume of reagent used the extent of the reduction can be calculated. The reagent is prepared by dissolving 250 g. of potassium carbonate in 700 ml. of hot water and adding 100 g. of potassium bicarbonate in small quantities; when solution is complete a solution containing 15 g. of copper sulphate is added and the solution diluted to 1 litre after cooling to 15°. The determination is carried out by adding 20 ml. of the fructose solution to 50 ml. of the reagent, and warming the mixture at 49° in a stoppered bottle for 2½ hours. The mixture is cooled and diluted to 100 ml.; it is filtered and, after rejecting the first 25 ml. of the filtrate, 50 ml. of the filtrate is acidified with sulphuric acid, potassium iodide added and the liberated iodine titrated with N/10 sodium thiosulphate. A blank titration on 50 ml. of the copper reagent is made. The quantity of fructose present in the 20 ml. of solution is given by the following formula:

$$x = \frac{n + 0.7}{0.26} \text{ mg.}$$

in which n is the difference between the blank and actual titrations. The presence of small quantities of glucose does not interfere with the determination. —S. Streptkoff, per *Quart. J. Pharm.*, 1937, 88.

Glucosum Liquidum (B.P.). n_{D20° , not less than 1.490. Ash, not more than 0.6%. Sulphur dioxide limit, 450 parts per million. By evaporation with water and drying at 90°, Glucosum Liquidum, *U.S.P. XII*, loses not more than 21% of its weight; ash, not more than 0.5%.

Determination of Glucose. 0.2 ml. of sample is added to 1.6 ml. of N/12 sulphuric acid and 0.2 ml. of a 10% solution of sodium tungstate; the mixture is well stirred, allowed to stand for five minutes and then centrifuged. 1 ml. of the clear liquid, equivalent to 0.1 ml. of blood, is heated with 1 ml. of Fehling's solution in a boiling water-bath for 30 minutes. The precipitate is separated by centrifuging, washed four times with 2 ml. of distilled water, and dissolved in 2 ml. of aqua regia, and the solution is made alkaline with ammonia and filtered. The filter is washed with water containing a little ammonia and the filtrate made up to 100 ml. Using 5 or 10 ml. of the solution the copper content is determined colorimetrically against a standard copper solution containing not more than 0.05 mg. of Cu. As some auto-reduction of the Fehling's solution takes place, a blank determination is made.—E. Lasausse *et al.*, *J. Pharm. Chim.*, Paris, ii/1936, 461.

Lactosum (B.P. Add. I). $C_{12}H_{22}O_{11} \cdot H_2O = 360.2$. A 10% w/v boiled solution has a specific rotation, determined at 20°, of +52° to +52.6°. Ash, not more than 0.1%. Tests for more soluble sugars, copper and acidity are included. Limit of matter soluble in 4 parts of alcohol (90%), 0.2% (limit of more soluble sugars). Lactosum, *U.S.P. XII*, should leave not more than 0.1% of ash, and should comply with tests for sucrose, dextrin and starch, and for dextrose.

Lævulosum (B.P.). $C_6H_{12}O_6 = 180.1$. Determined at 20° on a well-boiled 10% w/v aqueous solution, the specific rotation should be not less than -81°. Loss at 105°, not more than 5%. Sulphated ash, not more than 2.5%.

Mel Depuratum (B.P.). Sp. gr. 1.359 to 1.361. α_D for a 20% w/v decolorised solution, +0.6° to -2°, corresponding to a specific rotation of +3° to -10°. Ash, not more than 0.3% w/w. Artificial invert sugar is excluded by adding one drop of

resorcinol in hydrochloric acid solution to an evaporated ethereal extract, when no persistent cherry-red or brown-red colour should be produced. Mel, *U.S.P. XII*, has a sp. gr. of not less than 1.099 at 25° when diluted with twice its weight of water and should comply with tests for acidity, starch or dextrins, foreign colouring matter, azo dyes, and with the following test for artificial honey or added invert sugar:—

If 1 g. of honey be triturated with 20 ml. of ether in a mortar, filtered, the filtrate allowed to evaporate, and 1 drop of a 1% resorcin solution in hydrochloric acid added, a pink colour may form which disappears in half a minute, but an orange, cherry or brown-red colour must not be produced.

The Food and Drug Administration of the U.S. Dept. of Agriculture defined honey as the nectar and saccharine exudations of plants gathered, modified and stored in the comb by honeybees (*Apis mellifica* and *A. dorsata*). Was required to be lavorotatory and to contain not more than 25% of water, not more than 0.25% of ash, and not more than 8% of sucrose. Comb honey is contained in the cells of comb. Extracted honey is separated from the uncrushed comb by centrifugal force or gravity. Strained honey is removed from the crushed comb by straining or other means.—*S.R.A., F.D., No. 2, Rev. 5, Nov. 1936.*

Sucrosom (B.P.). $C_{12}H_{22}O_{11}=342.2$. Specific rotation in 10% *w/v* solution, +66° to +66.7°. Ash, not more than 0.05%. The absence of ultramarine should be indicated by the absence of unpleasant odour in one hour on the addition of dilute hypophosphorous acid to the clear, colourless, odourless solution. The *U.S.P. XII* determines the specific rotation on a 26% *w/v* solution of Sucrosom dried at 105°, and in a 200 mm. tube; it should not be less than +65.9°. The *Fr. Cx.* 1937 requires a 16.29% solution of the dry substance in a 2 dm. tube to have a rotation not less than 21.6°.

Sucrose may (in the absence of a polarimeter) be approximately estimated by heating 1 g. in 50 ml. of water, to which 10 drops of hydrochloric acid have been added, for half an hour on a water-bath. The solution is then cooled and neutralised with soda and made up to 100 ml. with water, and the invert sugar thus formed is estimated with Fehling's solution, 1 ml. of which is approximately equivalent to 0.005 g. of invert sugar, the calculation being on the basis that 360 of invert sugar represents 342 of cane sugar.

Determination of Invert Sugar in Cane Sugar. *U.S.P. XII* gives the following: Dissolve 20 g. sucrose in enough distilled water to make 100 ml.; filter if necessary. To 50 ml. of the clear liquid add 50 ml. of alkaline cupric tartrate solution: heat the mixture so that 4 minutes are required to bring it to boiling point and boil for 2 minutes. Add 100 ml. of cold recently boiled distilled water, and collect and weigh the precipitated cuprous oxide as follows. Prepare a Gooch crucible with an asbestos layer. Wash the asbestos with distilled water, followed successively by 10 ml. of alcohol and 10 ml. of ether, dry at 100° for 30 minutes and weigh the crucible. Filter the precipitated cuprous oxide through the crucible, wash the residue on the filter with hot distilled water, then with 10 ml. of alcohol, and then with 10 ml. of ether, and dry at 100° for 30 minutes. The weight of cuprous oxide does not exceed 0.112 g., corresponding to not more than 0.3% of invert sugar.

DIGITALIS

Digitalis Folium (B.P. Add. I). Should contain not more than 2% of foreign organic matter, lose not more than 8% at 100°, and leave not more than 5% of acid-insoluble ash. Digitalis, *U.S.P. XII*, contains not more than 2% of browned leaves, stems, flowers,

or foreign organic matter; acid-insoluble ash, not more than 5%; moisture not more than 6%; activity of 0.1 g. equivalent to not less than one U.S.P. Digitalis Unit (equivalent to the International Digitalis Unit of 1928). *Folium Digitalis*, *P. Helv. V*, consists of the leaves of *Digitalis purpurea* Linn., collected when dry, dried immediately at 40° and then for 30 minutes at 55° to 60°. Moisture, not more than 1%; ash, not more than 10%; acid-insoluble ash, not more than 4%. Powdered digitalis consists of the powder of the whole leaf prepared without any residue and is not standardised biologically or chemically.

Digitalis Lanata. The leaves of *Digitalis lanata* Ehrh. have become a regular article of commerce; their physiological activity has been investigated by a number of workers, and the results published show that they have an activity which is from two to five times as great as that of the leaves of *D. purpurea* Linn. The following characters enable the leaves to be distinguished from those of *D. purpurea*. The cells of both epidermises have anticlinal walls which are distinctly beaded. The non-glandular trichomes are uniseriate, consisting usually of from 10 to 14 cells; they are confined almost exclusively to the margins of the leaves. The paucity of the non-glandular trichomes causes the leaves to appear almost glabrous. Water pores occur singly or in groups consisting commonly of two pores.—T. Dewar, *Quart. J. Pharm.*, 1934, 331.

Constituents. Digitalis contains several glycosides, to which its physiological activity is due; of these, digitoxin, $C_{41}H_{64}O_{13}$, and gitoxin, $C_{41}H_{64}O_{14}$, have been obtained crystalline. A third glycoside, $C_{47}H_{72}O_{18}$, is described as having the composition of digitoxin with the addition of one molecule of dextrose; it is hydrolysed by the enzymes of the leaf into digitoxin and dextrose. The remaining glycosides have not been obtained in a state approaching purity; they are amorphous and more soluble than the crystalline glycosides. The latter are very sparingly soluble in water, but become more soluble in the presence of the amorphous glycosides. Distinctive names have been given to various mixtures, but only the two above-mentioned glycosides are definite substances. Gitalin and digitalein are mixtures of indefinite composition. Anhydrodigitalin is a crude form of gitoxin. Digitalis seeds do not contain digitoxin or gitoxin, but contain a closely-related glycoside, digitalinum verum, $C_{58}H_{88}O_{14}$, associated with a large proportion of water-soluble glycosides and two physiologically-inactive glycosides, digitonin, $C_{54}H_{86}O_{12}$, and gitonin, $C_{54}H_{86}O_{12}$. Digitalinum verum is not crystalline, but it forms crystalline derivatives. A standardised mixture of glycosides prepared from the seeds is known as digitalin, or digitalinum purum germanicum.

A preparation of the glycosides representing about 74% of the total activity of the original leaf may be obtained by extracting the leaf with benzene. The residue is extracted with alcohol, and benzene added followed by water. The aqueous-alcoholic layer is concentrated by means of chloroform followed by precipitation of the glycosides with light petroleum.—E. Hesse, W. Altner and J. Becher, *Klin. Wschr.*, 1933, 12, 1862.

Fresh concentrated infusions made with 20% alcohol or 0.1% chloroform water are essentially digitoxin preparations, whereas the fresh B.P. infusion is a digitalein preparation. Old concentrated infusions showed absence of therapeutic value in the filtrate with presence of toxic effects, the sediment being also toxic and possessing tonic effect.—K. Samaan, *Pharm. J.*, i/1921, 481.

Differentiation of Cardiac Glycosides. Digitoxin warmed for 3 minutes on a water-bath with 2 drops of glacial acetic acid and 10 drops of 0.3% w/v solution of vanillin in hydrochloric acid gives a permanent indigo blue colour; strophanthin-k gives a non-persistent deep blue precipitate, soluble in more acetic acid. Digitoxin in solution can be extracted before applying the test, by shaking with chloroform after rendering the solution alkaline with borax, drying and evaporating the chloroformic solution. Ouabain evaporated to dryness on a water-bath with 1 ml. of a solution of 0.1 g. of dimethylamino-benzaldehyde in 20 ml. of alcohol (95%) containing 4 drops of sulphuric acid gives a residue which is coloured violet when moistened with acetic acid. German digitalin under similar conditions gives an eosin-red colour. Digitoxin can be distinguished from German digitalin by addition of a reagent containing 1 drop of saturated bromine water in 20 ml. of sulphuric acid. German digitalin gives

a bright red, digitoxin shows no change.—J. A. Sanchez, *J. Pharm. Chim., Paris*, ii/1936, 549.

Stability. Digitalis dried at 100°F. immediately after harvesting is very stable in activity over a period of six years. Defatting slightly improves stability but air-tight and light-tight storage is entirely unnecessary. About 25% of the activity of the fresh drug may be lost during commercial drying.—L. W. Rowe and H. W. Pfeifle, *J. Amer. pharm. Ass.*, 1936, 855.

Martindale's Chemical Assay for Digitalis.

The determination of the value of a digitalis preparation (especially the tincture) by chemical means is fraught with considerable difficulties owing to many factors, e.g., the numerous glycosidal bodies contained, the fact that it is not possible to point to one glycoside as the potent constituent responsible for the activity of the drug, and again, the extraction of the substances in any degree of purity requires some analytical skill.

A tincture having by physiological tests a m.l.d. calculated as 0.75 ml. per 100 g. weight of frog was adopted as a standard.

In a paper read before the Pharmaceutical Society of Great Britain, Dec. 10, 1912, the results of examination were provided of a number of samples of leaves from various parts of the world (Great Britain, Germany, Italy, India, etc.). Almost all of these leaves gave tinctures of standard, or above standard, strength. (The paper was later published in a booklet entitled "*Digitalis Assay*.")

The colorimetric method devised was claimed by Martindale to give results equivalent to the physiological assay method based on the minimum lethal dose required to kill a frog and calculated to 100 g. body weight. The method requires some care in carrying out, as it is strictly quantitative. It is as follows:—

To determine whether a tincture is up to physiological test requirements (usually taken at m.l.d. = 0.75 ml. per 100 g. body weight of frog) mix 10 ml. of the tincture with 10 ml. of water, precipitate with 10% neutral lead acetate solution (about 3 ml.), adding a little diatomite. Allow to stand for 15 minutes, filter off on the pump and wash the precipitate slightly. Remove excess of lead from the filtrate with 10% sodium phosphate solution (about 2 ml. required) and filter. Add a little calcium carbonate (about 0.2 g.) to the filtrate (to prevent possible hydrolysis of the glycosides), and evaporate to dryness on a water-bath. Add about 2 g. of dry washed sand to the residue and extract with chloroform five times by thorough trituration, using about 10 ml. on each occasion. Filter and evaporate the chloroformic solution and extract the residue with warm water on the water-bath, using 10 ml. and 5 ml. and again employing sand. Filter, evaporate to dryness in a porcelain basin, extract the residue again with cold chloroform to purify it (about three or four quantities of 5 ml. each, using dry sand and triturating thoroughly with a small pestle) and filter. Evaporate the combined chloroformic liquors and dissolve the residue in 4 ml. of glacial acetic acid. Mix 0.1 ml. of this acetic solution with 1 ml. of sulphuric ammonium molybdate reagent in a 5 × 1 cm. test-tube and compare the depth of colour after five minutes with that produced with 10 ml. of the standard tincture, mounting the tubes on a little slab of plasticine and observing the colours by direct transmitted light using a white background. This coloration indicates the content of combined "active water-soluble" glycosides (probably including digitoxin). Further, if 0.1 ml. of the acetic solution be mixed with 0.5 ml. of glacial acetic acid, and this be layered upon 1 ml. of the sulphuric ammonium molybdate reagent, the typical blue ring showing presence of digitoxin should be formed.

Chemical assay processes used in the past centred on an estimation of the digitoxin—ignoring the bodies which are known to be readily soluble in water. That this was fallacious was shown by Ziegenbein, who found that leaves containing only 0.125% of this glycoside were twice as active as those containing 0.226%.

The above method includes the latter in addition to digitoxin or an allied body. The process includes a strong indication of digitoxin—either through the actual solubility of it in the repeated quantities of solvent (the amount of digitoxin in 10 ml. of tincture is extremely minute though sufficient to detect), or owing to the fact that digitoxin is soluble in the presence of the other glycosides and saponins. Results approximating the physiological m.l.d. results were obtained with the samples of tinctures referred to, even after 12 months' storage.

Tschirch confirms Ziegenbein's findings that the digitoxin content in digitalis leaves is not in proportion to the physiological activity. It was found that chloroform must be used repeatedly (eight shakings) to remove the entire active substances.

Absolute alcohol, acetone and amyl alcohol will exhaust leaves completely; chloroform, acetic ether and benzene partially. Ether and carbon tetrachloride do not extract the active substances at all. Acetone is especially good as yielding a colourless extractive. It can be used for assaying. (The use of acetone instead of chloroform in Martindale's method might yield interesting results.)—Tschirch & Wolter, *Schweiz. ApothZtg.*, 56, 469; per *Pharm. J.*, 1/1919, 219.

A comparison of the results of the biological assay and the colorimetric assay of Knudson and Dresbach on nine samples of leaves from 4 different species showed very close agreement. In the colorimetric process referred to, the infusion of the sample is precipitated first with lead acetate and then with sodium phosphate, and the filtrate is treated with sodium picrate. The colour produced is compared with that given by a standard solution of ouabain. In place of the latter, the authors used a solution of potassium dichromate.—B. J. Oikeloën and J. C. Timmer, *Pharm. Weekbl.*, 1931, 68, 820.

Method found unreliable.—J. A. C. Pinxteren, *ibid.*, 1932, 69, 4.

Only one out of fifteen samples assayed by the method of Knudson and Dresbach agreed with the biological (frog) assay.—F. J. Dyer, *Quart. J. Pharm.*, 1932, 172.

Digitalis Pulverata (B.P.). Adjusted for therapeutic administration to contain 10 units in 1 g. Loss at 100°, not more than 8%.

BIOLOGICAL METHODS OF ASSAY

The potency of powdered digitalis, *B.P.*, is determined by comparing each sample with a standard preparation of powdered digitalis which is distributed by the National Institute for Medical Research, Hampstead. The strength of the standard preparation has been carefully determined by comparison with the international standard digitalis powder. When an unknown sample has been compared with the standard preparation, its potency can then be expressed in terms of the international standard, that is to say, a statement can be made as to how much stronger or weaker the sample is than an average sample. To make this statement short, the international standard is arbitrarily described to contain 10 units of activity in 0.8 g.

Samples of powdered digitalis for therapeutic administration must be adjusted to contain 10 units per g. The maximum dose for repeated use is given as 0.1 g., or 1.5 grains, which is 1 unit, while the maximum single dose is 0.6 g., or 10 grains, which is 6 units. *B.P.* permits any method of assay to be used which is based on the effect of digitalis on cardiac muscle, or which gives results parallel to those obtained on cardiac muscle. *B.P.* suggests the frog method and gives instructions for performing it.

The Frog Method. The standard preparation and the unknown sample are extracted by a suitable method, for example tinctures of each are prepared with 70% alcohol. The tinctures, diluted with saline, are injected into two groups of frogs, which are as nearly as possible alike. The proportion of frogs killed in 24 hours by a given dose of the standard tincture and the proportion killed by a given dose of the unknown are then determined. Now, the relative potency of doses killing different proportions of frogs has been investigated on large numbers of frogs (Trevan, *Proc. roy. Soc.*, Ser. B, 1927, 101, 483) and a table showing these relative potencies is given in *B.P.* For

example, if the dose which kills 50% is taken as 100, then experiment has shown that the dose killing 30% is 87. By means of this table the potency of the unknown tincture is related to that of the standard. *B.P.* '32 requires that not less than 50 frogs be used in the comparison, since the standard deviation, that is to say, the error in two-thirds of the tests, will then not be greater than 10%. Likewise, the error in twenty-one out of twenty-two tests will not be greater than 20%.

The South African clawed frog or toad, *Xenopus laevis*, was found to respond more constantly than species of *Rana* to the digitalis series of drugs and is recommended for use in the biological assay of digitalis.—J. W. C. Gunn and D. Epstein, *Quart. J. Pharm.*, 1932, 180.

The Cat Method. The cat method differs from the frog method in that the minimum lethal dose for each cat is determined. Much confusion has arisen due to the incorrect use of this term; when a single dose of digitalis is injected into a frog, or of another drug into another animal, the animal either dies or survives, and it is impossible to say whether the dose was the minimal lethal dose or not; it is only possible to discover with accuracy the dose killing a certain proportion of animals, for none of which it may be minimal. The term L.D. 50 was introduced by Trevan to indicate the "mean lethal dose" which kills 50%. The expression "minimal lethal dose" should be reserved for those estimations in which the least amount of a substance necessary to kill is actually determined, of which the administration of digitalis to a cat is an example. As in the frog method both the standard preparation and the unknown sample of powdered digitalis are extracted by a suitable method; a 0.5% hot water infusion (90° for 15 minutes) is convenient. The extract is placed in a burette from which it is allowed to flow at a slow constant rate into the vein of an anæsthetised cat. The time of inflow is usually from 30 to 45 minutes, at the end of which period a concentration of the digitalis glycosides reaches the heart sufficient to produce arrest in systolic contraction. Throughout the experiment the cat is given artificial respiration to ensure that death does not occur from respiratory failure. The amount of extract administered up to the time of cardiac arrest is recorded, and a calculation made to determine the amount per kg. body weight. The figures for a series of cats are obtained both for the standard extract and for the unknown extract. *B.P.* 1932 requires that the standard extract be tested on 14 cats and the unknown on 6 cats; with these numbers the error of the estimation is then about the same as in the frog method. The test of the standard extract is not repeated each time an unknown extract is tested; it is sufficient to test the standard extract once a year, as cats do not vary in sensitiveness at different times. The average minimal lethal dose of a 0.5% hot water infusion of the international standard digitalis powder is 18.0 ml. per kg. cat. This corresponds to 90 mg. per kg. of the powder itself, but the figure varies according to the composition and amount of the

anæsthetic used. (See Macdonald, *Quart. J. Pharm.*, 1934, 182). While 90 mg. per kg. is the figure for cats under light anæsthesia with ether, 75 mg. per kg. is the figure for cats fully anæsthetised with ether.

Ether is an unsuitable anæsthetic for use in the assay of digitalis by the cat method in the tropics, but identical results are obtained when other anæsthetics are used, provided an uninterrupted and uniform depth of anæsthesia can be obtained without any side-effects on the circulation or respiration.—J. C. David and N. Rajamanickam, *Quart. J. Pharm.*, i/1934, 36.

HATCHER CAT UNIT. Hatcher defined the cat unit of powdered digitalis as the average minimal lethal dose per kg. Thus 1 cat unit of the international standard digitalis powder can be taken as 90 mg., but as seen from the foregoing paragraph, it depends on the amount of anæsthetic. Since 1 international unit is by definition the amount of activity in 80 mg. of the international standard digitalis powder, it may be said that the relation between the Hatcher cat unit and the international unit is 9 to 8, but the relation varies according to the technique of each laboratory.

POTENCY IN INTERNATIONAL UNITS. If the average minimal lethal dose for the 0.5% hot water infusion of the standard is found to be 16 ml. per kg., and if the figure for a similar extract of the unknown sample is 14 ml. per kg., then the potency of the unknown sample is $\frac{16}{14} = 1.14$ times that of the standard. If

the standard preparation is exactly equal to the international standard digitalis powder, then the unknown sample of powdered digitalis contains 11.4 units per 0.8 g.

Guinea-pig Method. The *B.P.* mentions the method of intravenous injection into guinea-pigs, which is in essentials similar to the cat method. It was introduced by Knaff-Lenz (*J. Pharmacol.*, 1926, 29, 407). Animals weighing 400 g. to 700 g. are anæsthetised with urethane, 1.75 g. per kg., injected under the skin. The digitalis extract, in concentration of 1.25%, is allowed to enter the jugular vein at constant rate. The experiment takes about 20 minutes. The method has the advantage that guinea-pigs vary less in sensitiveness to digitalis than cats, and a smaller number give the same accuracy. The *B.P.* '32, however, requires the same number of guinea-pigs as of cats.

Pigeon Method. When one of the cardiac glycosides is injected into the vein of a pigeon, it vomits in the course of 10 to 15 minutes, provided the dose is sufficient. (Hanzlik, *J. Pharmacol.*, 1929, 35, 363; also Burn, *ibid.*, 1930, 39, 221.) Powdered digitalis may be assayed by preparing a tincture and comparing it with a tincture prepared from the standard preparation. A dose of the order of 0.2 ml. of tincture per kg. is injected, the tincture being diluted so that the volume given to a bird weighing 300 g. is about 0.3 ml. The dose is given to each of a group of about twenty-five birds, and the proportion which vomit is observed. This proportion is determined both for unknown and for the standard. The relative potency of unknown and standard is then calculated from data previously obtained. (See Burn, *loc. cit.*)

United States Pharmacopœia XII Method. The frog method of the *U.S.P. XI* has now been replaced by a method using cats. The potency of 0.1 g. is required to be equivalent to not less than 1.0 *U.S.P.* Digitalis Unit when compared with *U.S.P.* Digitalis Reference Standard. One *U.S.P.* Digitalis

Unit is identical in potency with the International Unit as defined by the League of Nations' Commission. The 1942 Digitalis Reference Standard is identical, within the limits of biological error, in potency with the International Reference Standard. The Digitalis Reference Standard of the *U.S.P. XI* was found to be considerably stronger than previously stated and digitalis preparations of the *U.S.P. XI* had a corresponding higher potency. Tincture of digitalis *U.S.P. XI* was found to be 50% stronger than the tincture of the *U.S.P. X*, whereas it had been stated to be 25% stronger. The correction now made in the *U.S.P. XII* results in the fact that powdered digitalis and tincture of digitalis *U.S.P. XII* are weaker than the corresponding preparations of the *U.S.P. XI* and are 125% the strength of those of the *U.S.P. X*.

Dog Method. The use of dogs anaesthetised with Pentobarbital gives results similar to those obtained with frogs. By injecting intravenously the supernatant liquid of the tincture prepared from international powder the L.D. in dogs was shown to be 120 mg. of powder per kg. bodyweight, corresponding to twelve injections of 0.1 ml. of liquid per kg. Experiments with dogs and cats indicated that 0.62 g. and not 0.745 g. as stated, of the reference powder of *U.S.P. XI* is equivalent to 1 g. of international powder.—P. Blickensdorfer and H. A. McGuigan, *J. Amer. pharm. Ass.*, 1940, 101.

Chick Heart Method. A method for the assay of digitalis tincture or whole leaf preparations on the embryonic chick heart. The technique requires no elaborate equipment and no extensive training on the part of the operator and gives results which are at least as close to man as the chick assay.—R. A. Lehman and G. H. Paff, *J. Pharmacol.*, 1942, 75, 207.

Tabellæ Digitalis (*U.S.P. XII*). Contain powdered digitalis corresponding in potency to 95 to 105% of the labelled amount of powdered digitalis. Assayed by preparing a tincture from not less than 20 tablets and completing the assay biologically as described for tincture of digitalis. In the preparation of the tincture, the tablets are triturated with alcohol-water mixture (4:1) until disintegrated, the suspension is transferred to a hard glass-stoppered container and sufficient menstruum added so that 1 ml. may be expected to contain 1 *U.S.P.* unit. The mixture is then shaken mechanically for 22 to 26 hours at 20° to 30° so that the solid material is brought into fresh contact with the liquid phase. The suspension is then centrifuged and the tincture transferred to a well closed container, preserved under refrigeration and assayed within 30 days.

Tinctura Digitalis.

(a) *B.P.* 1 ml. must contain 1 unit of activity. The assay is performed by comparing the tincture with a tincture prepared from the standard preparation (*B.P.*) by one of the methods described for powdered digitalis. The tincture is usually prepared to contain more than 1 unit per ml.; it is then assayed and diluted to exactly 1 unit per ml. The supply of the International Standard Digitalis Powder (containing 1 unit of activity in 0.1 g.) having been exhausted, and a new powder (containing 1 unit in 0.08 g.) having been substituted, the *B.P. Add. I* makes the necessary adjustment for Tinctura Digitalis to possess 1 unit of activity (equivalent to the activity of 0.08 g. of the International Standard Digitalis Powder) in 1 ml. It may also be prepared by a maceration process.

(b) *U.S.P. XII.* The potency is such that, when assayed as directed, 1 ml. of the tincture possesses activity equivalent to 1.0 *U.S.P.* Digitalis Unit.

Digitalinum (*B.P.C.*). Possesses 80 units of activity in 1 g. (equivalent to the activity of 8 g. of the International Standard Digitalis Powder). Assayed biologically by the *B.P.* process for Digitalis Pulverata.

Kiliani Test for Digitalin. Ferric sulphate, 0.05 g., is dissolved in water, 1 ml., and sulphuric acid added to 100 ml. Employed as a mixing test (0.1 mg. of the glycoside is sufficient, dissolved in 0.2 ml. of glacial acetic acid), this reagent produces a pink coloration.

This test with digitoxin produces a brownish colour.

Assay. Digitalin is assayed by one of the methods described for powdered digitalis, in comparison with the standard preparation described in *B.P.* '32. Gage (*Quart. J. Pharm.*, 1933, 161) has found that unstandardised samples vary from 55 to 250 units per gramme. The dose given in *B.P.C.* for the standardised digitalin is $\frac{1}{2}$ to 1 grain for single administration; this corresponds to 2.5 to 5 units; it is injected subcutaneously or intramuscularly, being the only digitalis body which can be given in this way.

Digoxinum (*B.P. Add. V*). $C_{41}H_{64}O_{14}$ = 780.5. M.p. (with decomposition), 265°. α_D of 2% w/v solution in anhydrous pyridine (mercury light), +13.5° to +13.7°. Allied glycosides are limited by addition of sulphuric acid to a 0.1% solution in glacial acetic acid containing 0.01% w/v of ferric chloride, when the pure brown ring at the junction of the liquids should be free from red colour.

Digoxin can be given by mouth or by intravenous injection. Its potency has been determined by White (*J. Pharmacol.*, 1934, 52, 1) on various species. On the cat and the frog it has about one-quarter of the activity of ouabain, and probably contains about 2500 units per gramme. It exerts a cumulative effect when administered by mouth to cats or guinea-pigs.

Assayed biologically by the frog method, 0.6 mg. of digoxin was equivalent to 1 ml. of standard tincture, whereas when assayed by the cat method the equivalent amount was 0.4 mg. Administered orally to patients with auricular fibrillation, single doses of 1 to 2 mg. produced a rapid fall in ventricular rate, and the average subsequent daily dose found necessary was 0.5 mg. It is not necessary to administer digoxin by injection except in exceptional cases.—E. J. Wayne, *Clin. Sci.*, 1933, 1, 63.

Digitoxinum (*B.P.C.*). M.p., not below 240°. Loss at 100°, not more than 1%.

Keller-Kiliani (*Syn. Keller's Test for Digitoxin in the Leaves*). Shake 10 ml. of filtered infusion in boiling water, 1+20, in a separator for a few minutes with chloroform, 10 ml., add ether, 5 ml., and alcohol, 5 ml.; shake again and filter off the chloroform-ether solution through a filter moistened with chloroform. The liquid is evaporated and the residue dissolved in 3 ml. of acetic acid (96%). A drop of diluted solution of ferric chloride (1+19) is added, and the whole, in a narrow test-tube, is layered carefully upon sulphuric acid; at the point of contact of the two liquids a brownish-red zone develops, and over it a bluish-green zone.—*P.G. V*. It has been found in practice that the presence of chlorophyll hinders the coloration considerably.

The test may also be applied to the glycosidal substance *Digitoxin* thus:—Dissolve 0.001 g. in 3 ml. of glacial acetic acid, add a few drops of the ferric chloride solution and proceed exactly as described above.

Froehde's Test (sulphuric ammonium molybdate, see colorimetric method above). Ammonium molybdate, 1% w/v, in concentrated sulphuric acid used as a *mixing* test gives characteristic maroon colour with the water-soluble glycosides. Used as a layering test, it gives a characteristic blue ring.

There is no standard in *B.P.* '32 or in *U.S.P. XI* for digitoxin. Gage (*Quart. J. Pharm.*, 1934, 654) has examined a series of commercial samples in comparison with the standard preparation for powdered digitalis (*B.P.*) by two biological methods, the frog method and the guinea-pig method, and found that their activity varied from 900 to 1200 units per gramme. A pure crystalline sample of Digitaline Native had a potency of 1500 units per gramme.

Convallariæ Radix (*N.F. VII*). Tested by the *U.S.P. XII* method for Digitalis, the potency of 0.1 g. is equivalent to 3 *U.S.P.* Digitalis Units. Foreign organic matter, not more than 5% and acid-insoluble ash, not more than 6%.

Fluidextractum Convallariæ Radicis (*N.F. VII*). The potency of 0.1 ml. is equivalent to 2.75 to 3.25 *U.S.P.* Digitalis Units.

Activity of Tincture. Assays of commercial tincture by the frog method of the Russian Pharmacopœia and by the international cat method gave ranges of 10.5 to 17.6 units per ml. and 1.8 to 3.1 cat units per ml. respectively, with averages of 14.2 units and 2.1 cat units per ml. respectively.—S. I. Ordynskii, per *J. Amer. pharm. Ass.*, 1937, 348.

EPHEDRA

Ephedra (B.P.C.). Contains not less than 1.25% of total alkaloids calculated as $C_{10}H_{15}ON$. Assayed by the B.P.C. process as follows:—Add 200 ml. of ether-chloroform (3 : 1) to 20 g. of the powdered drug, and after 5 minutes, add 10 ml. of dilute solution of ammonia and 1 g. of anhydrous sodium carbonate, shaking frequently for four hours and standing overnight; after percolation to exhaustion with 100 ml. of ether-chloroform and then with ether, extract the percolates with N/3 hydrochloric acid, nearly neutralise the filtered acid extracts with N/1 sodium hydroxide, add 10 g. of anhydrous sodium carbonate and salt to saturation, and extract with ether; allow the decanted, filtered ether extracts to evaporate spontaneously, dissolve the residue in excess of N/10 sulphuric acid and back titrate with N/10 sodium hydroxide to methyl red.

An official method for the determination of total alkaloids in ephedra is described in *Official Methods of Analysis (A.O.A.C. 1940)*. 10 g. of powdered drug is shaken with 100 ml. of ether-chloroform (3 : 1) and after 5 minutes 5 ml. of 10% ammonia and 0.5 g. of anhydrous sodium carbonate added and shaken occasionally during at least 4 hours. An aliquot part of the clear liquid is extracted with 2% sulphuric acid which is then made ammoniacal, 5 g. of anhydrous sodium carbonate added and completely extracted with ether. The filtered ether extracts are evaporated on a steam-bath with aid of a fan, to a 5 ml. volume, an excess of standard sulphuric acid added, the ether evaporated and back titrated using bromothymol blue indicator. It seems that this process may give low results, due to the volatile nature of ephedra alkaloids when raised above very low temperatures.

The following method is suggested as yielding more consistent results than are obtainable with the B.P.C. method:—10 g. of the herb in No. 40 powder is shaken for 5 minutes with 200 ml. of a mixture of 1 volume of chloroform and 3 volumes of ether. Subsequently 10 ml. of 10% solution of ammonia and 1 g. of anhydrous sodium carbonate are added, the mixture shaken half-hourly for four hours, and then allowed to stand overnight. Next morning the mixture is transferred to a percolator and percolation continued with 100 ml. of the ether-chloroform mixture and then with ether until the alkaloid is completely extracted. The combined percolates are shaken with successive quantities of 40, 30, 20 and 20 ml. of N/3 hydrochloric acid and N/1 sodium hydroxide is added to the combined filtered acid extracts until nearly neutral. 10 g. of anhydrous sodium carbonate is added and sufficient sodium chloride to saturate the solution. The alkaline liquid is extracted with 20, 10, 10 ml. of chloroform followed by successive 5 ml. portions until the alkaloid is completely extracted. Three extractions are usually sufficient. The combined chloroform solutions are washed with 5 ml. of brine, the brine being washed with 5 ml. of chloroform which is added to the main bulk; 20 ml. of N/10 hydrochloric acid is pipetted into the bulked chloroform in a separator, shaken, and the chloroform run into a second separator, where it is washed with distilled water twice, the washings being added to the acid liquors in the separator. The combined acid liquors are titrated with N/10 sodium hydroxide to methyl red.—F. E. Rymill and C. A. Macdonald, *Quart. J. Pharm.*, 1937.

Ephedrina (B.P. Add. IV). $(C_{10}H_{15}NO)_2 \cdot H_2O = 348.3$. Contains 94 to 95% of the anhydrous base, determined by conversion to the hydrochloride and drying at 100°; the product has

a m.p. of 217° to 219° and α_D of the 5% w/v solution in water, -33° to -35° . M.p., without previous drying, 40° to 41° . Ash, not more than 0.1%. Ephedrina Anhydrosa (*B.P.C. Supp. III*), $C_{10}H_{15}NO = 165.1$, is assayed by the same process and contains not less than 98% of pure alkaloid. M.p., 33° to 37.5° , α_D of the hydrochloride obtained in the assay, -33° to -35° . Ephedrina, *U.S.P. XII*, is the synthetic or natural product and may be anhydrous or hydrated. The anhydrous substance contains not less than 98.5% of $C_{10}H_{15}NO$ and the hydrated substance not less than 94% $C_{10}H_{15}NO$. M.p., 34° to 40° , variation being due to differences in moisture content. α_D at 25° of the previously prepared hydrochloride -33° to -35.5° in 5% aqueous solution. Assayed by titration of the substance, dissolved in neutralised alcohol, against N/10 hydrochloric acid, using methyl red indicator. The label on the container must state whether Ephedrine *U.S.P. XII* is anhydrous or hydrated, and when the quantity of alkaloid in a preparation is stated, it is understood to be in terms of anhydrous ephedrine, unless otherwise stated.

Ephedrinæ Hydrochloridum (*B.P. Add. IV*). $C_{10}H_{15}ON, HCl = 201.6$. M.p., 217° to 219° . Specific rotation in 5% w/v aqueous solution, -33° to -36° . Loss at 100° , not more than 0.5%, and ash, not more than 0.1%. The salt of the *U.S.P. XII* is the natural or synthetically produced substance. After drying over sulphuric acid for 24 hours, contains 80 to 82.5% of anhydrous $C_{10}H_{15}ON$. Determined by extraction with successive portions of ether from a solution in saturated brine made alkaline with N sodium hydroxide. The combined ether extracts are washed with brine and then extracted with N/10 sulphuric acid and water. Excess acid is titrated with N/50 sodium hydroxide, using methyl red indicator. Loss over sulphuric acid for 24 hours not more than 2%. Ephedrinum hydrochloricum, *P. Helv. V*, by titration of the chloride, contains in the dried substance not less than 99.6% of $C_{10}H_{15}ON, HCl$.

Tabellæ Ephedrinæ Hydrochloridi (*N.F. VII*). Contain 91 to 109% of the labelled amount of ephedrine hydrochloride, including all tolerances. Assayed by dissolving in water, making alkaline with N/2 sodium hydroxide, extracting the alkaloid either by continuous extraction or by shaking with ether, finally extracting the washed ether solution with N/10 sulphuric acid and titrating the excess acid with N/50 sodium hydroxide, using methyl red indicator.

Ephedrinæ Sulphas (*B.P.C.*). $(C_{10}H_{15}ON)_2, H_2SO_4 = 428.3$. Specific rotation on a 10% w/v aqueous solution, -30° to -31.6° . Loss at 100° , not more than 0.5%. Ash limit, 0.1%. Ephedrinæ Sulphas, *U.S.P. XII*, dried over sulphuric acid for 18 hours and assayed as the hydrochloride, contains 75.5 to 77.3% of anhydrous ephedrine; a moisture limit of 2% is allowed.

Tabellæ Ephedrinæ Sulphatis (*U.S.P. XII*). Contain 93 to 107% of the labelled amount of $(C_{10}H_{15}NO)_2, H_2SO_4$, including all tolerances. Assayed by macerating a weighed quantity of powdered tablets with water and N/1 sulphuric acid for 2 hours, decanting the liquid through a filter, macerating the residue with a further quantity of water, decanting through the same filter and washing the flask and filter with water. After evaporation to a specified volume, the aqueous extract is saturated with sodium chloride, made alkaline with N/1 sodium hydroxide and extracted with ether. The combined ether

extracts are washed with water and the aqueous washings are extracted with a further quantity of ether which is added to the main ether extract. Most of the ether is removed at not more than 30°, an excess of N/50 sulphuric acid added, and the heating continued to remove the remainder of the ether and dissolve the alkaloid. After cooling, the excess N/50 sulphuric acid is titrated with N/50 sodium hydroxide, using methyl red indicator.

Quantitative methods for the determination of ephedrine in inhalants and tablets are described in *Methods of Analysis (A.O.A.C., 1940, 585)*. The ephedrine extracted with ether from ammoniacal solution is treated with excess standard sulphuric acid and back titrated with standard sodium hydroxide using bromothymol blue indicator and using standard indicator at pH 6.0 for comparison.

A colorimetric method for the determination of ephedrine in hypodermic tablets and injections is described. The alkaloid is extracted from ammoniacal solution with methylene chloride and after the combined extracts have been washed with saturated solution of sodium chloride, they are evaporated to dryness with a small quantity of benzoic acid. The residue is dissolved in water, 10% copper sulphate solution and 20% sodium hydroxide solution are added and the mixture is shaken with cyclohexane and allowed to separate. The tint of the upper layer is then determined under specified conditions and tables correlating the value of the red component to the ephedrine content are given.—N. L. Allport and N. R. Jones, *Quart. J. Pharm.*, 1942, 249.

Amphetamina (B.P.C. Supp. III). $C_9H_{13}N = 135.1$. Contains 95% to the equivalent of 100% of $C_9H_{13}N$. B.p., 200° to 203°, with slight decomposition. Sp. gr. at 25°, about 0.931. Residue on volatilisation on a steam-bath for 1 hour, not more than 0.1%. Assayed by titration with N/2 sulphuric acid using methyl red indicator.

Amphetaminæ Sulphas (B.P.C. Supp. III). $(C_9H_{13}N)_2 \cdot H_2SO_4 = 368.3$. Contains 72 to 73.5% of the base. Loss at 100°, not more than 1%. Assayed by extraction of the liberated base with ether, addition of excess N/2 sulphuric acid and after evaporation of the ether, back titration with N/2 sodium hydroxide using methyl red indicator.

The following method was used for the estimation of amphetamine in aqueous solution. The solution which is usually acid is neutralised with sodium hydroxide solution (10%) and 1 ml. added in excess. It is then extracted with successive quantities (25, 15, 10 and 10 ml.) of chloroform. Each chloroform extract is washed with the same quantity (5 to 10 ml.) of water, which is then shaken with a little chloroform. The whole of the chloroform used is combined and washed with another 5 ml. of water. The amphetamine is re-extracted from the chloroform by shaking with four successive portions of 10 ml. of N/2 hydrochloric acid. The acid extracts are evaporated to dryness on a water-bath, the residue dissolved in absolute alcohol, filtered into a tared flask, again evaporated to dryness, dried and weighed. Directions are also given for the estimation of amphetamine in viscera and urine. Tablets of amphetamine sulphate may be assayed by the following method. The crushed tablets are heated with 25 ml. of alcohol (50%) for a few minutes on a water-bath and the mixture filtered, the residue being washed with absolute alcohol. The alcohol is removed from the filtrate, evaporation to dryness being avoided, and the remaining liquid filtered into a separator, the filter being rinsed with acidified water. The filtrate is neutralised with sodium hydroxide solution (10%), 1 ml. in excess added, and the process completed by extraction with chloroform as described above.—E. T. Illing, *Analyst*, 1940, 3.

ERGOTA

Ergota (B.P. Add. VI). Should contain not more than 2% of foreign organic matter and not less than 0.2% of total alkaloids, calculated as ergotoxine, of which not less than 15% consists of

water-soluble alkaloids calculated as ergometrine. In the assay the drug in moderately fine powder is defatted by continuous extraction with light petroleum (b.p. 40° to 50°) and dried below 40° . After the addition of anæsthetic ether and dilute solution of ammonia, the drug is exhausted by continuous extraction with ether for about 5 hours. After filtering, washing the filter with ether and addition of acetone, the ethereal solution is extracted with 20, 10, 10 and 10 ml. of 1% tartaric acid solution. Dissolved solvent is removed from the mixed acid solutions at room temperature under reduced pressure, and the volume adjusted with water. 1 volume of the acid solution is mixed with 2 volumes of dimethylaminobenzaldehyde solution (0.125% in a cooled mixture of 65 ml. of sulphuric acid and 35 ml. of water to which 0.1 ml. of ferric chloride solution is added), the mixed solutions allowed to stand 5 minutes and the total alkaloids determined by matching against the blue-violet colour produced with a standard solution of ergotoxine ethanesulphonate similarly treated.

In the determination of the water-soluble alkaloids, an aliquot of the acid solution from the previous assay is made alkaline with dilute solution of ammonia and extracted with anæsthetic ether. The ethereal solution is washed with faintly ammoniacal water saturated with anæsthetic ether and subsequently extracted with 10, 5, 5 and 5 ml. of 1% tartaric acid solution. The water-insoluble alkaloids are then determined in the manner described above. The percentage of water-soluble alkaloids is calculated as ergometrine by multiplying by 0.538 the difference between the percentages of total alkaloids and water-insoluble alkaloids.

The normal total alkaloidal content of Spanish and Portuguese ergot ranges from about 0.2 to 0.3% and the average is about 0.25%. The proportion of water-soluble alkaloid calculated as ergometrine is about 20% of the total alkaloid but some analysts consider that a determination of water-soluble alkaloid is a very inaccurate measure of the ergometrine content and that the latter may be only 10% of the total alkaloid. Russian ergot is usually an inferior article containing a very small proportion of alkaloid and is unsuitable for official use except as a diluent for making prepared ergot.

Ergota, *U.S.P. XII*, contains not more than 4% of seeds, fruits, or other foreign organic matter, not more than 6% of moisture, and is standardised biologically (see below). *Secale cornutum*, *P.G. VI*, assayed by the process prescribed, contains not less than 0.05% of the water-insoluble alkaloids of ergot.

The use of a colorimeter is not essential. Dilute the solutions about ten times with water and compare the colours in Nessler glasses.—P. A. W. Self, *Pharm. J.*, i/1933, 245.

New alkaloids reported are ergocristine, isomeric with ergotoxine, and ergocristinine.—A. Stoll and E. Burckhardt, per *Analyst*, 1938, 54.

The effect of hot solvents on original ergot has been investigated. Ether, dichlorethylene, trichlorethylene and benzene have been found to extract the major portion of the alkaloids; light petroleum does not extract the alkaloids.

In the case of dichlorethylene and benzene, quantitative recovery of the alkaloids has been made, proving that the alkaloids are extracted and not destroyed by the solvents.—R. F. Corran and F. E. Rymill, *Quart. J. Pharm.*, 1935, 337.

60 mg. of water-soluble and 2 g. of water-insoluble alkaloids found per kg. of ergot as compared with the finding of Hampshire and Page that the water-soluble alkaloids amount to $\frac{1}{3}$ th and $\frac{1}{4}$ rd of the water-insoluble alkaloids.—E. Rothlin, *Arch. exp. Path. Pharmac.*, 1937, 184, 69.

Determination of Fat. Widely different results can be obtained in using light petroleum, according to the method applied. The *B.P.* test is unsatisfactory and should be made quantitative; the *B.P.C.* figure of 15 to 30% of fat is an under-estimate and 30 to 40% is closer to the true amount present.—W. A. N. Markwell, *Pharm. J.*, i/1941, 32.

BIOLOGICAL METHODS OF ASSAY

There is no specific biological method of estimating ergometrine which is now recognised as probably the most important alkaloid for clinical purposes.

U.S.P. XII Method. Ergot is assayed by the cock's comb method. When an extract of ergot is injected into the breast muscles of a cock, the ergotoxine present causes a darkening of the colour of the comb. The darkening is first seen in the hindmost part of the comb, and, according to the amount of ergotoxine, spreads forward. An unknown sample of fluidextract is compared with a 0.05% solution of ergotoxine ethanesulphonate in 1% tartaric acid solution. From 5 to 10 cocks of about 2 kg. weight are used for the comparison, and receive doses varying from 0.25 ml. to 0.5 ml. The combs of birds which have been injected with the sample are then compared with those injected with the standard.

Ergot is required to possess a potency per gramme equivalent to not less than 0.5 mg. of the official standard ergotoxine ethanesulphonate.

Rabbit Uterus Method. A method which has been widely used for estimating the ergotoxine present in ergot extracts was described by Broom and Clark (*J. Pharmacol.*, 1923, 22, 59). When strips of the uterus of a rabbit are suspended in a bath of oxygenated Ringer's solution at 37°, the strips contract when adrenaline is added to the bath. If ergotoxine is added to the bath, the power of the strip to respond to adrenaline soon disappears, according to the concentration of ergotoxine and the time it is allowed to act. To estimate the ergotoxine present in an extract, two strips are cut from the same piece of uterine muscle and suspended in baths placed side by side. The response to a given dose of adrenaline is recorded for each strip, and when fresh Ringer's solution has been placed in each bath a dose of ergotoxine is added to one bath and a dose of the unknown extract is added to the other. After an interval which must be the same for each bath, and may be 8 or 10 minutes, the dose of adrenaline is added again. The response is now reduced, and is reduced by the greatest amount in that bath in which most ergotoxine is present. By making observations on many pairs of strips, a figure can be obtained for the amount of ergotoxine in the extract, but the method is tedious and is subject to a large error.

A biological method for the assay of ergot is described in *Methods of Analysis (A.O.A.C., 1940, 627)*.

Ergota Præparata (B.P. Add. VI). Contains from 0.19 to 0.21% of total alkaloids of ergot, calculated as ergotoxine of which not less than 15% consists of water soluble alkaloids,

calculated as ergometrine. Yields on extraction with light petroleum (b.p., 40° to 50°), not more than 5% of fat.

Extractum Ergotæ (B.P.C.). When freshly prepared, contains 0.5% of total alkaloids, calculated as ergotoxine. *Extractum Ergotæ, N.F. VII*, when assayed by the *U.S.P. XII* method, has a potency per g. equivalent to 1.6 mg. of ergotoxine ethanesulphonate reference standard.

Extractum Ergotæ Liquidum (B.P.). Assayed by extracting the diluted extract, made ammoniacal, with anæsthetic ether, shaking the ether with tartaric acid solution and proceeding as for the assay of Ergota, it contains when freshly prepared 0.06% *w/v* of total alkaloids, calculated as ergotoxine; after storage it contains not less than 0.04% *w/v*. *Fluidextractum Ergotæ, U.S.P. XII*, is of the same strength as the *U.S.P. XI* preparation, 1000 ml. being obtained from 1000 g. of recently ground drug, but no assay process is prescribed.

The colour with *p*-dimethylaminobenzaldehyde is given also by the pharmacologically inactive alkaloids ergotinine and ψ -ergotinine which are present in liquid extract of ergot. About 60 to 70% of the total alkaloids consists of ergotoxine and the standard of 0.05% of total alkaloids corresponds therefore to about 0.03% of ergotoxine.—*Rep. of Pharmacopœia Sub-Committee on Ergot, October, 1931.*

Stability. When diluted in a mixture it will lose all activity in 2 or 3 days, but if stored in completely filled unopened bottles the loss of activity during six months is inappreciable.—B. A. Bull, *Pharm. J.*, i/1933, 317.

If the liquid extract is kept under ordinary dispensary conditions for more than six weeks the strength is likely to fall below the *B.P.* limit. The keeping properties are much improved by storage in full bottles in the dark, and the deterioration in an ice-chest is very slow.—E. M. Smelt, *Quart. J. Pharm.*, 1933, 399.

On dilution with water, it yields a precipitate which contains the greater part of the alkaloids. The loss in strength is about 30% in 10 to 14 days. When dispensed with other substances the rate of loss of activity differs with different extracts but is usually more rapid. Ferric chloride greatly increases the loss of activity.—E. F. Hersant and W. H. Linnell, *Pharm. J.*, ii/1933, 3.

Ergometrina (B.P. Add. I). $C_{19}H_{23}O_2N_3 = 325.2$. M.p. of the lower melting form, on the air-dried substance and raising the temperature 4° per minute, 162° to 164°, with decomposition; m.p. of the higher melting form, on material dried *in vacuo* for one hour at 140°, 212° with decomposition. α_D of 1.5% *w/v* solution in dehydrated alcohol, using the air-dried substance and calculated on the substance with the associated solvent removed, +40° to +43° (sodium light), and +60° to +63° (mercury light). Associated solvent determined by heating *in vacuo* for one hour at 140°.

Ergonovinæ Maleas (U.S.P. XII). $C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4 = 441.5$. Loses not more than 2% when dried over sulphuric acid and then contains 98% of $C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$. M.p., with decomposition, about 167°; α_D at 25° determined on an aqueous solution containing 1% of dried material, +48° to +57°. Assayed by extraction with ether from an ammoniacal alcohol solution, previously diluted with saturated brine, removal of the ether, addition of excess N/50 hydrochloric acid and back titration with N/50 sodium hydroxide, using bromophenol blue indicator.

Tabellæ Ergonovinæ Maleatis (U.S.P. XII). Contain 90 to 110% of the labelled amount of $C_{12}H_{13}N_3O_2 \cdot C_4H_4O_4$, including all tolerances. Assayed by maceration of a weighed quantity of powdered tablets with alcohol, filtering and colorimetric estimation using dimethylaminobenzaldehyde in an aliquot of the filtrate.

Determination of Ergometrine. Ergometrine differs from ergotamine and ergotinine by being moderately soluble in water whereas the other alkaloids are insoluble. It can be extracted from an ethereal solution of total alkaloids by shaking with water. The colour produced by 0.538 g. of ergometrine in the dimethylaminobenzaldehyde test (using Allport and Cocking's reagent) is equivalent to that produced by 1 g. of ergotamine base. In the following assay the total alkaloids and the water-insoluble alkaloids, both expressed as ergotamine, are determined; the difference gives the water-soluble alkaloids as ergotamine, and the latter figure multiplied by 0.538 gives the equivalent of ergometrine. **Total alkaloids.**—Shake 60 ml. of the ethereal solution with successive quantities of 10, 10, 5, and 5 ml. of a 1% w/v solution of tartaric acid. Mix the acid solutions and warm gently in a current of air. Cool and dilute to 30 ml. with water. Mix 1 ml. with 2 ml. of solution of dimethylaminobenzaldehyde and allow to stand for five minutes. Mix 1 ml. of solution of ergotamine ethanesulphonate with 2 ml. of solution of dimethylaminobenzaldehyde and allow to stand for five minutes. Determine the ratio of the colour intensities by comparing them in a suitable colorimeter. **Water-insoluble alkaloids.**—Shake the remaining 60 ml. of ethereal solution with successive quantities of 20 ml. of water made faintly alkaline to litmus with ammonia, until 1 ml. of the separated aqueous liquid gives no blue colour when mixed with 2 ml. of solution of dimethylaminobenzaldehyde. Shake the ethereal solution with successive quantities of 10, 10, 5, and 5 ml. of a 1% w/v solution of tartaric acid. Mix the acid solutions and remove the ether by gentle warming in a current of air. Cool, dilute to 30 ml. with water, and compare with solution of ergotamine ethanesulphonate as described in the assay for total alkaloids. The difference between the results of these two assays multiplied by 0.538 gives the water-soluble alkaloids calculated as ergometrine. The figure includes also any ergometrinine.—C. H. Hampshire and G. R. Page *Quart. J. Pharm.*, 1936, 60.

A colorimetric method for the determination of ergometrine and ergotamine in hypodermic tablets and injections.—N. L. Allport and N. R. Jones, *Quart. J. Pharm.*, 1942, 246.

Assay of Ergotocin (Ergometrine). A comparison of six different methods of assay—polarimetric, colorimetric, cock's comb, isolated rabbit's uterus, post-partum dog's uterus and post-partum human uterus. The isolated rabbit's uterus method, preferably supplemented by the clinical method, is preferred. The oxytocic action typifies ergotocin and eliminates ergotamine and ergotamine, and the prolonged rhythmic contractions distinguish it from histamine and tyramine which give only brief responses. The post-partum dog's uterus is less sensitive than the human. The Broom and Clark method is inapplicable since ergotocin has no inhibitory action on adrenaline and its own action on the isolated rabbit uterus is diminished by the previous application of ergotamine or ergotamine.—E. E. Swanson *et al.*, *J. Amer. pharm. Ass.*, 1935, 836.

Precipitation with picric acid can be used for demonstrating the alkaloids of the ergotamine group in ergometrine. Ergometrine can be assayed by titration with hydrochloric acid using bromophenol blue as indicator.—F. Reiners, *Quart. J. Pharm.*, 1938, 253.

Assay of Ergot and its Liquid Extract for Ergometrine.

The method previously described by Hampshire and Page for the separate determinations of the water-soluble and the water-insoluble alkaloids of ergot has been modified and applied to the liquid extract.

ASSAY OF LIQUID EXTRACT.

(a) **Total Alkaloids.** 25 ml. of liquid extract in a Watkins extractor is made alkaline with ammonia and extracted with pure ether for six hours. Transfer the aqueous-ether liquid to a separator and shake out with 4 × 5 ml. of 1% tartaric acid. Warm the mixed acid liquids, dilute to 25 ml. and determine colorimetrically with standard ergotamine ethanesulphonate solution.

(b) **Water-insoluble Alkaloids.** 25 ml. of liquid extract made alkaline to litmus with ammonia is extracted with 3 × 40 ml. of pure ether. Shake the mixed ethereal solutions with 5 × 40 ml. of water previously made alkaline with ammonia and saturated with pure ether. Extract the washed ether solution

with 4 × 5 ml. of 1% tartaric acid, remove ether by warming, dilute to 25 ml. and compare with the ergotoxine solution as before.

(c) *Water-soluble Alkaloids*. This is determined by difference and expressed as ergometrine by multiplying by the factor 0.538.

ASSAY OF ERGOT. Extract 10 g. in moderately fine powder with light petroleum (40° to 50°) in a continuous extractor to remove fat and dry the residue below 40°. Mix in a dish with pure ether to form a soft paste and stir with 2 ml. of strong ammonia. Replace the residue in the extractor and extract for 5 hours with 100 ml. of pure ether. Filter and wash the filter and flask with pure ether until 120 ml. is obtained. Shake the ethereal solution with 20, 10, 10 and 10 ml. of 1% tartaric acid, warm to remove ether, cool and dilute with water to 50 ml.

For *total alkaloids* mix 2 ml. of dimethylaminobenzaldehyde reagent with 1 ml. of the alkaloidal solution and determine colorimetrically against standard ergotoxine ethanesulphonate solution. For *water-insoluble alkaloids* take 25 ml. of the alkaloidal solution, make alkaline with ammonia and extract with 40, 30, 30 and 20 ml. of pure ether. Then shake the ether with 5 × 40 ml. of alkaline water saturated with ether and extract this washed ethereal solution with 10, 5, 5 and 5 ml. of 1% tartaric acid. Warm the mixed acid solutions to remove ether, cool, dilute to 25 ml. with water and determine colorimetrically as before.—C. H. Hampshire and G. R. Page, *Quart. J. Pharm.*, 1938, 57.

The determination devised by Hampshire and Page for powdered ergot is extended for the liquid extract and tablets.—C. K. Plycart, *J. Ass. off. agric. Chem.*, 1937, 28, 566.

While methods are available for the separation of water-soluble ergot alkaloids from water-insoluble, there is none for the separation of ergometrine from its inactive isomer, ergometrinine. The following method is therefore presented:—25 ml. of a solution of the alkaloids in 1% tartaric acid solution is made faintly alkaline to litmus with ammonia, 35 ml. of ether (U.S.P.) added and the mixture shaken for 3 minutes. The aqueous layer is washed with two 35-ml. portions of ether contained in two other separating funnels, and finally run into a 250-ml. beaker. The process is repeated with six 10 ml. portions of water containing sufficient ammonia to give it a pH of 10, and the aqueous extracts combined in the beaker. The combined extract is warmed slightly to remove any dissolved ether, the volume adjusted to 100 ml. and then assayed colorimetrically. By this method, about 98 to 99% of the ergometrine is obtained together with about 5% of ergometrinine, the ergometrine giving slightly high results. By applying the method to a mixture of water-insoluble alkaloids only, it was found that the second ether wash contained 5.7% of the total alkaloids used, and the third ether only 0.2%. This indicates that, in the Hampshire-Page assay process, about 6% of the water-insoluble alkaloids may be present in the aqueous extract.—D. C. Grove, *J. Amer. pharm. Ass., Sci. Edn.*, i/1941, 260.

A new method for the separation of ergometrine from other alkaloids in ergot and the liquid extract has been worked out by Allport and Porter in which a solution of the mixed alkaloids in chloroform containing 1.0% v/v of ethyl alcohol is added to a 2% solution of antimony trichloride in chloroform.

ASSAY OF LIQUID EXTRACT. To 10 ml. in a separator add 1 ml. of 10% ammonia and 25 ml. of methylene chloride, shake, separate the lower layer and repeat the extraction 6 times with 25 ml. of methylene chloride or until extraction is complete. Distill off the solvent, dry the residue by two evaporations with 5 ml. of acetone, dissolve in 25 ml. of special chloroform containing 1.0% v/v of ethyl alcohol and filter through cotton wool into a beaker containing 25 ml. of the special chloroform with 5 ml. of 2% w/v antimony trichloride in chloroform, stir, wash the flask, etc., with a further 20 ml. of the special chloroform, allow to stand one hour, and collect the precipitate in an ignited asbestos Gooch crucible, washing the beaker and precipitate with 20 ml. of a 0.2% w/v antimony trichloride solution in the special chloroform. Triturate the contents of the crucible in a beaker with 2 ml. of 95% alcohol, add 30 ml. of 15% tartaric acid solution warmed to 40°, allow to stand $\frac{1}{2}$ hour and dilute the solution with the asbestos to 50 ml. Take 1 ml. of the liquid and determine the ergometrine colorimetrically with 2 ml. of *p*-dimethylaminobenzaldehyde reagent in a Lovibond tintometer or against standard ergotoxine solution.

ASSAY OF ERGOT. Remove the fat from 10 g. in No. 60 powder by percolation with cold light petroleum (40° to 50°) and dry below 40°. Mix slowly in a mortar with 7.5 ml. of strong solution of lead subacetate, triturate for five

minutes and percolate with 250 ml. of a mixture of 9 volumes of pure ether and 1 vol. of methylene chloride until 2 ml. of the percolate gives no blue colour in the *p*-dimethylaminobenzaldehyde reaction. Evaporate the percolate to dryness, dry the residue with acetone and proceed as described in the process for the liquid extract.

Results given range from 0.003% in Russian ergot to 0.180% in a sample of ergot of *Festuca* grass, and from 0.004% in a liquid extract made by the B.P. 1914 process from Russian ergot to 0.036% in a liquid extract made by the B.P. 1932 process from an ergot giving 0.06% of alkaloid by the B.P. assay.—N. L. Allport and G. V. Porter, *Quart. J. Pharm.*, 1938, 96.

The methods of the B.P. '32, of Hampshire and Page and of Allport and Porter are examined and criticised and a new method using acetone as solvent is described.—C. Daglish and F. Wokes, *Quart. J. Pharm.*, 1939, 451.

The Hampshire-Page method of assay requires eight hours' extraction in a Soxhlet apparatus to ensure complete extraction, whereas the acetone-ammonia method of Wokes and Daglish is quicker and gives a higher yield of alkaloids.—S. A. Schou and M. Tønnesen, *Dansk Tidsskr. Farm.*, 1940, 14, 33.

Neither continuous extraction of ergot with methylene chloride nor shaking ergot with a menstruum of ammoniacal acetone completely extracts the alkaloids, whereas continuous extraction with anæsthetic ether for four to five hours does give complete extraction. During the last process, about 2% of the alkaloids are decomposed.—C. H. Hampshire and M. W. Partridge, *Quart. J. Pharm.*, 1941, 116.

In the Hampshire and Partridge ether-extraction process, it appeared that about 97% of the alkaloids were extracted in the first hour. It would be advisable to remove the ether after that time and continue the extraction with a fresh supply, to avoid a large amount of decomposition due to heating the alkaloids for five hours.—W. H. Linnell, *Quart. J. Pharm.*, 1941, 177.

The experimental error of the colour matching in ergot assays is a serious obstacle to further advance. The lowest estimate of the experimental error when using the colorimeter is 2%.—F. Wokes, *Quart. J. Pharm.*, 1941, 178.

A rapid method for the assay of ergometrine and ergotoxine is described. The total alkaloids are extracted by maceration and suction percolation with ammoniacal acetone, followed by a similar treatment with a mixture of ether and an aqueous suspension of magnesium oxide. After transference of the total alkaloids to an aqueous solution of tartaric acid, the ergotoxine is separated by extraction with ether after adjusting the reaction of the aqueous solution to pH 5.5. The ergometrine which remains in the aqueous layer is transformed to a solution of smaller volume and both alkaloids are finally determined colorimetrically. The method has also been applied to liquid extract of ergot.—N. L. Allport and N. R. Jones, *Quart. J. Pharm.*, 1941, 106.

Methods of assay are described in which the ergotoxine and ergometrine groups of alkaloids are separated and determined.—S. A. Schou, M. Tønnesen and I. Bennekou, per *Quart. J. Pharm.*, 1939, 119.

An assay process is described for ergot and liquid extract of ergot in which ergometrine is completely separated from ergotoxine-like alkaloids.—E. E. Swanson *et al.*, *J. Amer. pharm. Ass., Sci. Edn.*, 1941, 255.

Ergometrine may be prepared by the following process:—1 kg. of defatted powdered ergot is moistened with 1.5 litres of absolute methylated alcohol (5% methyl alcohol) to which 50 ml. of 0.88 ammonia has been added. The wet material is extracted in a percolator with absolute methylated alcohol. After about 3 litres of percolate have been collected, the extract is evaporated to about 200 ml. in a vacuum, the water-bath temperature being kept at 40° to 45°.

To this concentrate are added about 300 ml. of 0.25 N sulphuric acid (sufficient to render the reaction faintly acid to congo-red), and the distillation is continued until all the residual alcohol has been removed. Fat separates as the content of alcohol decreases. The fluid is then chilled at about 2°, when most of the fat becomes hard enough to be removed mechanically.

After filtration through paper, the acid aqueous solution, measuring 200 to 250 ml., is treated with saturated aqueous sodium carbonate solution (about 10 ml.) until the reaction is very faintly alkaline to litmus. It is then filtered from the flocculent precipitate which is produced, made alkaline by the addition of more saturated sodium carbonate solution (about 20 ml.) and extracted five times with 50 ml. quantities of chloroform. The chloroform extract is freed from watery droplets, carried over mechanically, by shaking with a small quantity of

anhydrous sodium carbonate, filtered, and evaporated in a vacuum (water-bath at 45° to 50°). As the solution is concentrated, crude crystalline ergometrine separates. The volume is reduced to from 5 to 10 ml. After keeping at 2° for an hour the crude ergometrine is collected on a filter and washed with a little chloroform. Suitable solvents for the recrystallisation of ergometrine are benzene, acetone, ethyl acetate, and methylethyl ketone; after adequate recrystallisation, e.g., from methylethyl ketone, it melts and decomposes at 161° to 162°. The yield from defatted Spanish ergot was 0.2 g. per kg.—H. W. Dudley, *Pharm. J.*, i/1935, 709.

The purest specimen of ergometrine obtained, exhibiting an E value of 185 and melting at 164°, gave a colour by the chemical test equivalent to that produced by 1.78 times its weight of ergotoxine base. Ergometrine in aqueous tartaric acid solution shows an absorption band in the ultra-violet region with a maximum at 316 m μ . The same band is exhibited by solutions of ergotoxine. The colour produced when ergometrine is submitted to the *p*-dimethylamino-benzaldehyde test is spectroscopically identical with that produced by ergotoxine under the same conditions.—N. L. Allport and Sydney K. Crews, *Quart. J. Pharm.*, 1935, 447.

Ergotoxina (B.P.C.). Specific rotation, determined on a 2% *w/v* solution of the anhydrous substance in chloroform, not less than -180°. On drying *in vacuo* at 90° it loses not more than 5%. At 190° to 200° the dry substance decomposes with evolution of gas. Ash limit, 0.1%.

Ergotoxinæ Ethanosphonas (B.P. Add. I). Specific rotation, determined on a 4% *w/v* solution of the anhydrous substance in acetone and water (2:1 by volume), +119° to +122°, and of the separated anhydrous base, the same as specified for Ergotoxina. The anhydrous salt contains acid equivalent to 16 to 16.7% of ethanesulphonic acid. Loses *in vacuo* at 90° to 100° not more than 5%. Ash limit, 0.1%.

Ergotamine. Ergotamine is less active biologically than ergotoxine but is approximately equivalent when examined colorimetrically. If used as a standard in the biological assay of ergot, 60% of the observed readings gives a close approximation of the ergotoxine content. A similar correction should be applied in the colorimetric assay of ergot owing to the biological inactivity of ergotinine.—E. Lozinski, G. W. Holden and G. R. Driver, *Quart. J. Pharm.*, 1933, 395.

Ergotinine is included in the *Fr. Cx.* 1937.

Ergotaminæ Tartras (U.S.P. XII). α_D at 25° of 0.4% solution in chloroform, -125° to -155°. Darkens on heating at 177° and decomposes at 184°. A test for foreign substances is included.

Tabellæ Ergotaminæ Tartratis (U.S.P. XII). Contain 90 to 110% of the labelled amount of $(C_{33}H_{35}N_5O_6)_2 \cdot H_2C_4H_4O_6$, including all tolerances. In the assay, a weighed quantity of powdered tablets is first extracted twice with light petroleum and the light petroleum rejected. The residue is then extracted 4 times with chloroform saturated with ammonia, each chloroform extract being passed through a filter and, finally, the residue is transferred to the filter and washed with chloroform. The combined chloroform extracts are washed with water and the aqueous washings are extracted with a further quantity of chloroform. After removal of almost all the chloroform, the alkaloidal residue is evaporated with alcohol and finally dried *in vacuo* over sulphuric acid and weighed.

Histaminæ Phosphas Acidus (B.P. Add. I). $C_5H_9N_3 \cdot 2H_3PO_4$ = 307.2. M.p., 130° to 133°, after sintering at 127°. Loss in a vacuum desiccator, not more than 1%. Histaminæ Phosphas, U.S.P. XII, dried at 100°, loses not more than 1.5%; m.p. about 130°.

EXTRACTUM FELLIS BOVINI

Extractum Fellis Bovini (B.P.). Gives no precipitate on addition of 2 vols. of alcohol (90%) to 1 vol. of a 1% *w/v* aqueous solution.

Tabellæ Extracti Fellis Bovis (U.S.P. XII). Contain an amount of the extract corresponding, in content of bile acids determined as cholic acid, to not less than 40% of the labelled amount of extract, including all tolerances. Assayed by triturating a weighed quantity of powdered tablets with alcohol (80%), passing the alcohol through a filter and making the solution up to volume. The bile acids are determined by diluting the solution, removing the alcohol from 1 ml. of the dilution *in vacuo* at room temperature, and adding water, 1% aqueous solution of furfural, sulphuric acid-water mixture (10 : 13), and heating at 70° for 8 minutes followed by immersion in ice-water for 2 minutes. The blue colour produced is compared with a standard solution of U.S.P. cholic acid reference standard similarly treated.

Sodii Tauroglycocholas (B.P.C. Supp. IV). When prepared from ox bile consists of a mixture of $C_{26}H_{44}O_7NSNa$ and $C_{26}H_{42}O_6NNa$ with small proportions of sodium taurodeoxycholate and sodium glycodeoxycholate. When prepared from pig bile contains compounds of taurine and glycine with hyodeoxycholic acid. Yields not less than 70% of total bile acids. Acid value of total bile acids from the assay, not greater than 145. Sulphated ash, 13 to 17%. Assayed by hydrolysis by refluxing with 15% sodium hydroxide solution for 12 hours, dilution and extraction of the filtered liquid and washings with ether, evaporation and drying at 100°.

FERRUM

Ferri Carbonas Saccharatus (B.P.). Assayed for ferrous iron content by titration of a phosphoric acid solution, diluted with warm water and 25% *w/v* sulphuric acid, with N/10 potassium dichromate using diphenylamine as indicator. Ferrous iron content, as $FeCO_3$, not less than 50%. The N.F. VII preparation is made with lactose and sucrose instead of glucose; it is assayed by titration against N/10 ceric sulphate using ortho-phenanthroline as indicator, and contains not less than 15% of $FeCO_3$. Ferrum Carbonicum cum Saccharo, P.G. VI, contains from 9.5 to 10% of iron. Ferrum oxydatum cum Saccharo, P.G. VI, contains from 2.8 to 3% of iron.

The iodate method is suitable for the assay of the saccharated iron compounds of the B.P. and B.P.C. and of ferrous lactate. Ferrous iron may be titrated with accuracy by iodate in the presence of liquid glucose, acacia, tragacanth, sucrose, invert sugar in small amounts, lævulose, dextrose, lactose, glycerin, lactic acid and citric acid. Invert sugar in great excess produces a small error. The method is unsatisfactory in the presence of liquorice, marsh-mallow, quinine and aqueous extract of cochineal.—G. J. W. Ferrey, *Quart. J. Pharm.*, 1935, 344.

Assay. The following method, using ceric sulphate, gives more accurate results than the official method. Digest about 2 g. accurately weighed, with 8 ml. of cold 25% *w/v* sulphuric acid until the mass has disintegrated. Dilute with 20 ml. of water and titrate with N/10 ceric sulphate using phenylanthranilic acid as internal indicator. Phenylanthranilic acid may be prepared by heating for an hour under a reflux condenser a mixture of bromobenzene 3.5 g., anthranilic

acid 3 g., anhydrous potassium carbonate 3 g., cuprous chloride 0.1 g., and nitrobenzene 15 g. The product is steam-distilled to remove the nitrobenzene and the crude acid precipitated by acidifying the hot liquid with dilute hydrochloric acid. The acid is dissolved with sodium hydroxide, the solution filtered and again precipitated with hydrochloric acid, and the crystals purified by reprecipitation and recrystallisation from alcohol. The indicator solution is prepared by dissolving 0.11 g. of phenylanthranilic acid and 0.3 g. of anhydrous sodium carbonate in 100 ml. of water. It becomes faintly pink on long standing but remains satisfactory as an indicator.—C. G. Lyons and F. N. Appleyard, *Quart. J. Pharm.*, 1937, 343.

Pilula Ferri Carbonatis (B.P.). Contains not less than 20% of ferrous iron, calculated as FeCO_3 , determined by digestion with phosphoric acid and water, addition of sulphuric acid and titration with N/10 potassium dichromate, using diphenylamine indicator.

Massa Ferri Carbonatis (U.S.P. XII). Contains 36 to 41% of FeCO_3 ; determined by titration in acid solution with N/10 ceric sulphate, using orthophenanthroline indicator. **Pilulae Ferri Carbonatis, U.S.P. XII,** determined similarly, contains in each pill not less than 0.06 g. of FeCO_3 .

Ferri et Ammonii Citras (B.P.). Contains from 20.5 to 22.5% of Fe. The assay by warming with 30 parts of water and 2 parts of sulphuric acid, oxidising the cooled solution with N/10 potassium permanganate, allowing interaction to proceed for 3 minutes with 30 parts of hydrochloric acid and 4 parts of potassium iodide, and titrating the liberated iodine with N/10 sodium thiosulphate, replaces the B.P. '14 method of weighing the ignited residue. **Ferri et Ammonii Citrates, U.S.P. XII,** should contain from 16.5 to 18.5% of Fe, and is assayed by interaction of a solution with hydrochloric acid and potassium iodide during 15 minutes. The *Fr. Cx.* 1937 substance, by re-ignition with nitric acid, contains 24 to 26% of iron oxide. **Ferrum citricum ammoniatum, P. Helv. V,** is assayed iodometrically and should contain from 17 to 18% of iron.

Ferri et Ammonii Citras Viridis (B.P.C.). Fe content determined as for **Ferri et Ammonii Citras**, from 14 to 16%. **Ferri et Ammonii Citrates Virides, U.S.P. XII,** contains 14.5 to 16% of Fe.

Ferri et Ammonii Citro-Arsenis (B.P.C. Supp. IV). Contains not less than 14% of Fe, and 1.3 to 1.5% of As_2O_3 . Assayed for iron by the process described for **Ferri et Ammonii Citras**, subtracting the volume of N/10 iodine required, for an equal weight, in the assay for arsenic trioxide from the volume of N/10 sodium thiosulphate; and assayed for arsenic trioxide by interaction, in presence of sodium bicarbonate, with excess N/10 iodine during 7 mins. and back titration with N/10 sodium thiosulphate.

Ferri et Mangani Citras (B.P.C.). Assayed by the method for **Ferri et Ammonii Citras**, should contain not less than 14% of Fe, and not less than 7% of Mn by the following process of the B.P.C.: 0.1 g. boiled till a clear solution is produced with 25 ml. of dilute nitric acid, is heated with 12 ml. of N/10 silver nitrate and 1 g. of ammonium persulphate for 30 seconds after oxidation commences; the cooled solution is titrated with N/100

the iodate method of Heisig. Ferrous chloride mixed with soft paraffin and enclosed in glyco-gelatin capsules is best determined by ceric sulphate.—G. J. W. Ferrey, *ibid.*, 351.

The following assay is recommended by the Sub-Committee on Inorganic Chemicals of the Committee on General Chemistry:—Dissolve about 1 g., accurately weighed, in 20 ml. of a 25% *w/v* aqueous solution of sulphuric acid, in a stoppered flask. Add 6 ml. of strong solution of iodine monochloride and 60 ml. of hydrochloric acid; titrate with M/20 potassium iodate until the solution becomes light brown in colour; add 5 ml. of chloroform and continue the titration until the chloroform becomes colourless. Each ml. of M/20 potassium iodate is equivalent to 0.02317 g. of FeCl_2 .—(*British Pharmacopœia Commission Report*, No. 14, September 1939.)

Syrupus Ferri Phosphatis (B.P.C.). By the *B.P.* titanous chloride titration method used for *Syrupus Ferri Phosphatis Compositus*, an iron content equivalent to from 1.7 to 1.9% *w/v* of $\text{Fe}_3(\text{PO}_4)_2$ should be indicated.

Syrupus Ferri Phosphatis Compositus (B.P.). Contains iron equivalent to from 0.85 to 0.95% *w/v* of anhydrous ferrous phosphate, $\text{Fe}_3(\text{PO}_4)_2$, and calcium equivalent to from 1.3 to 1.5% *w/v* of tricalcium phosphate. Assayed for iron by diluting a weighed portion with water, adding a few drops of hydrochloric acid and a 2% potassium permanganate solution until a transient pink colour is produced throughout the solution; after addition of more hydrochloric acid and sodium bicarbonate, N/10 titanous chloride solution is added until the commencement of the titration is indicated by the production of a blue colour on adding one drop of the titration liquid to one drop of potassium ferricyanide solution; the ferric iron is then titrated with N/10 titanous chloride using ammonium thiocyanate solution as indicator. Calcium is determined by well diluting a weighed portion, adding citric acid, and boiling, making just alkaline with ammonia, then adding acetic acid and, to the boiling solution, excess of ammonium oxalate solution; after boiling gently on a sand-bath for 2 hours, the precipitate is collected, washed and ignited with sulphuric acid.

Assay of Syr. Ferri Phosph. Co. The following assay, based on the method of Bradbury and Edwards (*J. Soc. chem. Ind., Lond.*, 1940, 96) is stated to overcome the difficulties of the official method. To about 20 g. of the syrup in a glass-stoppered separating funnel, add 1 ml. of sulphuric acid (50% *w/v*) and sufficient N/10 potassium permanganate to produce a transient pink. Adjust the volume to 40 ml., add 3 ml. of ammonium thiocyanate solution (40% *w/v*), and titrate with mercurous nitrate solution, rotating the funnel gently throughout. When the solution becomes brownish and obscures any red colour, add 20 ml. of ether and shake for thirty seconds. Run off the aqueous layer into a second separator, leaving any precipitated mercury in the first. Shake the contents of the second separator with 15 ml. of ether for thirty seconds; reject the aqueous layer. Add the second ethereal layer to the first, wash the second separator with 50 ml. of water, and add this to the mixed ethereal extracts. Shake the mixture well for twenty seconds to dissolve any mercury, and continue the titration, with shaking after each addition, until the red colour disappears. The mercurous nitrate solution is approximately N/10 in nitric acid (5% *w/v*). To standardise, place 10 ml. of N/10 ferric alum solution, 1 ml. of sulphuric acid (50% *w/v*), 29 ml. of water and 3 ml. of ammonium thiocyanate solution (40%) in a separator and titrate with the mercurous nitrate solution exactly as described in the assay process.—F. R. Bradbury, K. C. Chatterjee and E. G. Edwards, *Quart. J. Pharm.*, 1940, 297.

The pharmacopœial method for titration of iron with titanous chloride, owing to the instability of the reagent and the necessity for storing it in an inert

atmosphere, is inconvenient where only occasional determinations are required. The method described is rapid and convenient and requires little manipulation. Iron in the ferric state can be precipitated quantitatively by means of 8-hydroxyquinoline at pH 5 to 5.3 without interference from the other constituents of compound syrup of ferrous phosphate, Easton's syrup and many other B.P.C. iron syrups and preparations. Oxidation to ferric iron is effected by boiling with a definite amount of nitric acid and solution of ammonium citrate is added to act as a buffer and keep the calcium in solution. The most suitable temperature for precipitation of iron is 80° to 90°. Manganese and magnesium in the presence of iron may also be determined by this method. *Method*.—Accurately weigh 10 to 15 ml. of syrup of ferrous phosphate and heat to boiling with an equal weight of water and 2.0 ml. of nitric acid. Add 200 ml. of water and 20 to 30 ml. of solution of ammonium citrate solution (B.P. lead test solution). Heat to 80° to 90° and add 10 ml. of a 5% alcoholic solution of oxine. Stir, allow to stand for 2 to 3 minutes, filter through a tared sintered glass crucible, wash well with hot water and dry at 100° for 1 hour. 1 g. of precipitate = 0.1145 g. of Fe or 0.2444 g. of $\text{Fe}_2(\text{PO}_4)_3$. Alternatively, where small amounts of precipitate are concerned, it may be advantageous to use a volumetric method of estimation. The iron-oxine complex, containing 0.01 g. to 0.015 g. of Fe, is filtered and washed in the usual manner and is then dissolved on the filter in 20 ml. of hot hydrochloric acid-water mixture (1 : 1). The solution is sucked through into a stoppered bottle and the crucible washed with cold water, the total volume in the bottle being made up to about 130 ml. Excess N/10 bromide-bromate is added and the mixture allowed to stand for 5 minutes. 20 ml. of phosphoric acid is added to prevent the liberation of iodine from potassium iodide by the ferric iron present. Finally, 1 g. of potassium iodide is added and the liberated iodine titrated immediately with N/10 sodium thiosulphate. 1 ml. N/10 bromine = 0.000465 g. of Fe.—G. J. W. Ferrey, *Quart. J. Pharm.*, 1942, 255.

Assay for Calcium. The following method is much quicker and less troublesome than the pharmacopoeial determination, and the results obtained are in good agreement. Calcium is most completely precipitated as oxalate from a solution at pH 4.4 to 4.6 (green to blue-green with bromocresol green). The calcium solution is brought to this pH by taking 10 to 20 ml. of the sample, adding an equal volume of water and 4 ml. of nitric acid, boiling to oxidise iron and destroy colouring matter, then adding 20 ml. of ammonium citrate solution and diluting to 150 ml. The solution is then boiled, tested with bromocresol green, and treated with 50 ml. of ammonium oxalate solution, boiled gently for 15 minutes and filtered hot by suction through a tared sintered glass crucible. The precipitate is then thoroughly washed with cold water, dried at 100° for about 1 hour and weighed as $\text{Ca}(\text{COO})_2 \cdot \text{H}_2\text{O}$.—G. J. W. Ferrey, *ibid.*, 1942, 264.

Syrupus Ferri Phosphatis cum Quinina et Strychnina (B.P.). Should contain iron equivalent to from 1.62 to 1.98% w/v of $\text{Fe}_2(\text{PO}_4)_3$, 1.04 to 1.2% w/v of anhydrous quinine, and 0.022 to 0.027% w/v of strychnine. Assayed for iron by the titanous chloride titration method; for quinine, by adding sodium citrate to a dilution of the syrup and sodium hydroxide solution and extracting with chloroform; from the weight of the dried residue of total alkaloid the weight of strychnine is subtracted; the residue of total alkaloid is then dissolved in N/1 hydrochloric acid and an equal volume of saturated sodium chloride solution and extracted five times with 5-minute shakings with chloroform; the chloroform liquids are shaken with water and ammonia solution, evaporated, alcohol added, evaporated and dried at 100°; the residue is then washed with three 2 ml. portions of (2 : 1) ether and light petroleum (b.p., 50° to 60°) saturated with strychnine, evaporated with alcohol and, after drying at 100°, the residue of strychnine is weighed. The B.P. Add. VI includes **Syrupus Ferri Phosphatis cum Strychnina** which complies with the

above standards with the omission of quinine, and which is to be supplied when Easton's syrup is ordered.

Determination of Iron in Easton's Syrup. The disadvantages of the official method are overcome by applying the method of Bradbury and Edwards (*J. Soc. chem. Ind., Lond.*, 1940, 96) for the direct titration of iron with mercurous nitrate in the presence of thiocyanate ions. The method is as follows:—To about 12 g. of sample add 1 ml. of sulphuric acid (50% w/v), followed by N/10 potassium permanganate drop by drop until a transient pink colour is produced throughout the solution. Adjust the volume to 40 ml., add 10 ml. of ammonium thiocyanate solution (40% w/v) and titrate with mercurous nitrate solution in a glass-stoppered bottle, which is rotated throughout. Near the end-point a black precipitate is coagulated by shaking with 10 ml. of chloroform, and the titration then continued until the red colour just disappears. The mercurous nitrate solution is approximately N/10 in 5% w/v nitric acid. It is standardised by means of N/10 ferric ammonium sulphate, using a 40% w/v solution of ammonium thiocyanate.—F. R. Bradbury, K. C. Chatterjee and E. G. Edwards, *Quart. J. Pharm.*, 1940, 297.

Hydrogen peroxide is a more satisfactory oxidant for iron than potassium permanganate in the thiocyanate determination. The red colour can be made stable for several minutes, depending on the amount of peroxide used, and the faded colour may be restored if necessary by the addition of more peroxide. Too much peroxide may cause a yellowish interfering colour due to oxidation products of thiocyanate.—C. A. Peters *et al.*, *Industr. Engng Chem. (anal. Edn.)*, 1939, 502.

Determination of Quinine and Strychnine. The assay of the B.P. for strychnine is unsatisfactory with old samples of the syrup, owing to impure strychnine being obtained. In order to obtain pure strychnine, dissolve this residue in 10 ml. of N/1 hydrochloric acid and filter through a 9 cm. filter paper into a separator. Wash the flask and filter paper with three further quantities of 5 ml. of N/1 hydrochloric acid and then with 25 ml. of a saturated solution of sodium chloride. Repeat the extraction of the filtered liquid by shaking with five successive quantities of 25 ml. of chloroform and continue the B.P. process of separation to the same stage as above and weigh the residue, which should be almost white.—N. Evers and W. Smith, *Quart. J. Pharm.*, 1936, 397.

A tentative method for the separation of quinine and strychnine in liquid preparations is described in *Methods of Analysis (A.O.A.C. 1940, 592)*. The extracted and weighed mixed alkaloids are dissolved in 50 ml. of 10% sulphuric acid, 5 ml. of potassium ferrocyanide solution (4%) added drop by drop, and after standing for some hours, filtered and the precipitate washed with 5% sulphuric acid. The precipitate is immediately treated with ammonia solution and chloroform, the solution extracted with chloroform and the alkaloids transferred to 20% sulphuric acid. The ferrocyanide precipitation and extraction with chloroform is repeated, and the chloroform evaporated, the residue of strychnine dried and weighed and checked by titration to methyl red indicator. The combined two acid filtrates and washings from the ferrocyanide precipitation is made ammoniacal, extracted with ether-chloroform, and the quinine finally dried at 120° to 130°. The quinine may be checked by dissolving in alcohol, adding 1 ml. in excess of the quantity of N/50 sulphuric acid necessary to produce a yellow colour to bromo-cresol purple indicator, evaporating to low bulk, cooling and collecting the separated quinine sulphate, washing with water and back titrating the filtrate and washings.

Ferri Sulphas (B.P.). $\text{FeSO}_4 \cdot 7\text{H}_2\text{O} = 278.0$. By titration with N/10 potassium permanganate, it should contain not less than 99% of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. The U.S.P. XII salt contains from 54.36 to 57.07% of anhydrous ferrous sulphate, corresponding to not less than 99.5% of the heptahydrate.

Tabellæ Ferri Sulphatis (U.S.P. XII). Contain 95 to 110% of the labelled amount of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, including all tolerances. Assayed by dissolving a weighed quantity of powdered tablets in recently boiled and cooled water and dilute sulphuric acid, filtering from insoluble matter, and titrating immediately with N/10 ceric sulphate using orthophenanthroline indicator.

Ferri Sulphas Exsiccatus (*B.P. Add. VI*). Contains not less than 77% of FeSO_4 .

Ferrum (*B.P. Add. I*). The fine bright wire should have a diameter of about 0.1 mm. and contain not more than 200 parts of arsenic per million. Much of the wire on the market contains more arsenic than the *B.P.* allows.

Determination of Iron. Small proportions of iron can be determined by means of ammonium nitrosophenylhydroxylamine (*syn.* Cupferron), $\text{C}_6\text{H}_5\text{N}(\text{NO})\text{ONH}_2$. The reagent is used as a 5% solution in water, and should be filtered if necessary. The reagent becomes dark-coloured on long storage, and the solution also gradually becomes darker but keeps satisfactorily for several days. The solution containing about 0.1 g. of iron in 150 ml. to 200 ml. is strongly acidified with hydrochloric acid, and the reagent solution added with constant stirring until a white precipitate of nitrosophenylhydroxylamine begins to form. The brownish-red precipitate of the iron compound is collected, washed several times with dilute hydrochloric acid containing a few drops of the reagent, then with diluted ammonia solution (5% *w/v* of NH_3) to remove excess Cupferron and convert the iron to ferric hydroxide, and, finally, ignited. Each gramme of Fe_2O_3 is equivalent to 0.699 g. of Fe.

Ferrum Redactum (*B.P.*). Contains not less than 80% of metallic iron. Assayed by interaction with a hot copper sulphate solution and titration of the filtered and acidified liquid with N/10 potassium permanganate. **Ferrum Reductum**, *U.S.P. XII*, should contain not less than 90% of metallic iron; interaction at boiling-point with corrosive mercuric chloride is used for assaying, followed by adjustment to volume and titration of an aliquot part of the filtered liquid with N/10 potassium permanganate. The *Fr. Cx.* 1937 substance by similar assay is of the same standard.

Ferrum pulveratum, *P.G. VI*, contains not less than 97.6% of iron, and **Ferrum redactum**, *P.G. VI*, should contain not less than 96.5% of iron or not less than 90% of metallic iron. **Ferrum reductum**, *P. Helv. V*, contains not less than 90% of metallic iron; it is assayed by interaction with a boiling solution of mercuric chloride and titration of the filtrate with potassium permanganate solution.

Assay of Reduced Iron. The copper sulphate method for the determination of metallic iron in reduced iron has been shown to yield inaccurate and variable results. The modification of the Wilner-Merck process suggested yields consistent results which reflect the actual content of metallic iron in the sample. The method recommended is as follows:—To approximately 0.5 g. of sample in a clean, dry 100 ml. graduated flask add 2.5 g. of mercuric chloride (sulphide-free) and about 50 ml. of recently boiled and cooled distilled water. Boil gently for 20 minutes (avoiding excessive frothing) with frequent shaking, make the volume up to 100 ml. with recently boiled and cooled distilled water, cork the flask and cool. When cold, adjust the volume to 100 ml., shake well, allow the precipitate to settle, filter rapidly into a clean, dry conical flask, pipette 50 ml. of the filtrate into 100 ml. of dilute sulphuric acid in which 2 g. of manganese sulphate has been dissolved, and titrate with N/10 potassium permanganate solution.—Hartley, Linnell, Read and Rolfe, *Q. J. Pharm.*, 1935, 100.

Determination of Traces of Lead and Copper in Medicinal Iron Preparations. To 2 g. of sample in a 350 ml. hard glass flask add 5 ml. of water and 10 ml. of sulphuric acid. Heat gently, add slowly 10 ml. of 30% hydrogen peroxide (100 vols.), and boil. Add more hydrogen peroxide as necessary to oxidise the organic matter completely, as indicated by the absence of charring when all the excess water has been boiled off. Cool, add 50 ml. of water and 10 ml. of hydrochloric acid, and boil until a clear solution is obtained. Cool and add a solution of 10 g. of citric acid in 50 ml. of water and

30 ml. of strong solution of ammonia. Cool and neutralise to litmus paper with dilute solution of ammonia, adding a further 10 ml. of the dilute ammonia solution. Transfer to a separator and extract three times with 10 to 15 ml. portions of a 0.1% *w/v* solution of diphenylthiocarbazone in chloroform. Each extract is washed in a second separator with about 20 ml. of water, transferred to a small flask and the chloroform evaporated. Add 0.5 ml. of sulphuric acid to the residue and destroy organic matter by heating with a few drops of nitric acid, then remove nitric acid by adding a few drops of water to the cooled solution and heating until white fumes appear. The solution contains all the Cu and Pb. Dilute with water, add 1 g. of citric acid and 4 g. of ammonium acetate, and, when solution is complete, make slightly alkaline with ammonia and dilute with water to 100 ml. To determine the Cu, transfer 25 ml. to a Nessler glass, neutralise to litmus with glacial acetic acid, adding 2 ml. in excess, dilute to 100 ml. with water and add 1 ml. of a 0.1% *w/v* solution of dithio-oxamide in alcohol (95%). Compare the colour produced with that obtained with a solution containing 1 g. of ammonium acetate and a suitable quantity of standard copper sulphate solution containing 0.00001 g. of Cu per millilitre. The amount of copper solution used should not exceed 6 ml. otherwise a smaller amount of the original solution must be used. The Pb is determined by transferring 25 ml. of the original solution to a Nessler glass, adding 1 ml. of 10% *w/v* KCN solution and a little dilute ammonia, diluting to 50 ml. with water, adding 0.1 ml. of 10% sodium sulphide solution and matching in the ordinary way against the B.P. dilute solution of lead PbT, using an auxiliary solution containing 1 g. of ammonium acetate, 1 ml. of 10% *w/v* potassium cyanide solution and the same amount of Cu as is known to be contained in the primary solution. The amount of standard lead solution used must not exceed 10 ml.—N. L. Allport and G. H. Skrimshire, *Quart. J. Pharm.*, 1932, 460.

Traces of lead may be separated from considerable quantities of iron by extracting the iron, as ferric chloride, with ether from solution in 25 to 27% *w/v* hydrochloric acid.—A. D. Powell and G. F. Hall, *Quart. J. Pharm.*, 1932, 454.

Minute amounts of copper in the presence of iron and certain other metals can be determined colorimetrically by means of diethyldithiocarbamate and extraction with carbon tetrachloride.—L. A. Haddock and Norman Evers, *Analyst*, 1932, 495.

FILIX MAS

Filix Mas (B.P.). Contains not more than 2% of other organic matter. Crystals of calcium oxalate should be absent, and it should yield not more than 6% of ash and 2% of acid-insoluble ash. *Aspidium*, *U.S.P. XII*, on extraction with ether, shaking with barium hydroxide solution, acidifying with hydrochloric acid, extracting with ether, evaporating and drying the residue at 100°, yields not less than 1.5% of crude filicin; acid-insoluble ash, not more than 3%. *Rhizoma Filicis*, *P. Helv. V*, contains not less than 1.8% of crude filicin when determined by applying the usual baryta process to the residue obtained after percolation of the drug with ether.

Extractum Filicis (B.P.). Contains from 24 to 26% *w/w* of filicin. Sp. gr., not less than 1.000. n_{D40° , not less than 1.492. Determined by the B.P. method by shaking 5 g. in 40 ml. of ether with 100 ml. of barium hydroxide solution; 87 ml. of the separated filtered aqueous liquid (= 4 g. of extract) is acidified with hydrochloric acid, and extracted with 30, 20 and 15 ml. portions of ether; the filtered and evaporated ether extracts are dried at 100° and weighed as filicin. *Oleoresina Aspidii*, *U.S.P. XII*, yields not less than 24% of crude filicin. Sp. gr., not less than 1.00 at 25°.

Extractum Filicis, *P.G. VI*, should contain not less than 25% o crude filicin.

Assay of male fern by estimating the phloroglucosides with silver.—*Pharm. J.*, 1930, 321.

Biological Assay. The principles of an assay of extract of male fern is described, and on the basis of about 1000 earth worms a characteristic curve has been determined showing the relation between doses of extract and mortality.—F. Schönhayder, *Quart. J. Pharm.*, 1939, 975.

FENICULUM

Foeniculum (B.P.). Contains not more than 2% of other organic matter and yields not more than 12% of ash. The *N.F. VII* allows a limit of 4% of foreign organic matter for Foeniculum, and not more than 1.5% of acid-insoluble ash. Fructus Foeniculi, *P.G. VI*, is required to yield not less than 4.5% of volatile oil.

The Food and Drug Administration of the U.S. Dept. of Agriculture defined fennel seed as the dried fruit of cultivated varieties of *Foeniculum vulgare* Hill. Required to contain not more than 9% of total ash, and not more than 2% of ash insoluble in hydrochloric acid.—*S.R.A., F.D. No. 2, Rev. 5, Nov. 1936.*

Oleum Foeniculi (B.P.C.). Using a crystal of congealed oil of anise to induce crystallisation, the f.p. (by the *B.P.* method employed for Oleum Anisi) is not below 3°. Sp. gr., 0.960 to 1.000. α_D , +4° to +24°. n_{D20° , 1.525 to 1.550. The *U.S.P. XII* uses a small crystal of the substance being tested or rubs the inner walls of the tube to induce congelation in determining the f.p. of Oleum Foeniculi, which should not fall below 3°. Sp. gr., 0.953 to 0.973 at 25°. n_{D20° , 1.5280 to 1.5380. α_D at 25°, +12° to +24°.

FORMALDEHYDUM (LIQUOR)

Liquor Formaldehydi (B.P.). Contains 37 to 41% w/v of CH_2O . Sp. gr., 1.080 to 1.095. Estimated by heating on a water-bath with hydrogen peroxide and N/1 sodium hydroxide and back titrating the excess of alkali with N/1 sulphuric acid to phenolphthalein, a blank titration being performed. Liquor Formaldehydi, *U.S.P. XII*, should contain not less than 37% w/v of H·CHO; bromothymol blue indicator is used for the titration.

Soluté Officiel de Formaldéhyde, *Fr. Cx.* 1937, is assayed by the following method:—5 ml. of 1% solution and 35 ml. of potassium iodo-mercurate solution are shaken for five minutes with 20 ml. of sodium hydroxide solution; after 15 minutes, and while cooling, the mixture is acidified with dilute hydrochloric acid, the precipitated mercury dissolved in 20 ml. N/10 iodine and back titrated with N/10 sodium thiosulphate; a content of not less than 35% should be indicated. Formaldehyd solutus, *P.G. VI*, should contain at least 35% of H·CHO, determined by the following method:—About 1 g. of solution, accurately weighed, is placed in 100 ml. flask containing 2.5 ml. of water and 2.5 ml.

of N/1 potassium hydroxide. After shaking, make up to 100 ml. 10 ml. of the solution is now mixed with 50 ml. of N/10 iodine and 20 ml. of N/1 potassium hydroxide added. After 15 minutes, add 10 ml. of dilute sulphuric acid, and titrate with N/10 sodium thiosulphate. For every 0.1 g. of formaldehyde solution, at least 23.3 ml. of N/10 iodine is required, so that for the saturation of the excess iodine at most 26.7 ml. of N/10 sodium thiosulphate is necessary. This represents a content of at least 35% formaldehyde (1 ml. of N/10 iodine = 0.001501 g. of formaldehyde, using starch as indicator).

Formaldehydum solutum, *P. Helv. V*, should contain 35 to 36.5% of $\text{H}\cdot\text{CHO}$ and is assayed as follows:—Dilute about 6 g. with water to 100 ml.; to 25 ml. add 3 drops of thymolphthalein and neutralise with N/1 sodium hydroxide to produce a blue colour. Add a freshly prepared solution of 6.5 g. of solid sodium sulphite in 25 ml. of water made neutral to thymolphthalein with N/1 sodium hydroxide, shake gently and titrate with N/1 hydrochloric acid until the solution is completely decolorised. Each millilitre of N/1 HCl is equivalent to 0.03002 g. of $\text{H}\cdot\text{CHO}$.

In the assay process of *P. Helv. V* the direction to neutralise the sodium sulphite solution with alkali is incorrect. It should be neutralised to thymolphthalein with N/1 hydrochloric acid.—L. Rosenthaler, *Schweiz. ApothZtg*, 1935, 617.

The specification of the Association of British Insecticide Manufacturers (Bulletin No. 82: Ministry of Agriculture and Fisheries) requires Liq. Formaldehyde to be a colourless solution, neutral or faintly acid, containing not less than 36% nor more than 40% w/v formaldehyde.

In dilute solutions formaldehyde may be determined by admixture with potassium cyanide solution, addition to acidified silver nitrate solution, adjusting to volume, filtration and back titration of an aliquot with N/10 ammonium thiocyanate solution, a blank titration omitting the test solution being made.

Formaldehyde and acetaldehyde can be distinguished and determined, when mixed, with dimedone (dimethyldihydroresorcinol).—M. V. Jonescu and H. Slusanschi, *Bull. Soc. Chim.*, 1933, 53, 909.

Determination of Formaldehyde in Tablets. A colorimetric method of estimating formaldehyde and paraformaldehyde in tablets, using Schiff's reagent. The results obtained from commercial samples varied from 0.021 g. to 0.002 g. in commercial formaldehyde and menthol tablets weighing 0.791 g. and 0.69 g. respectively. An average content appears to be about 0.01 g.—N. Evers and C. M. Caines, *Yearb. Pharm.*, 1921, 321.

Paraformaldehydum (B.P.C.). By the oxidation method used for Liquor Formaldehydi, it contains not less than 95% of $(\text{CH}_2\text{O})_3$. Ash, not more than 0.1%. The *U.S.P. X* also required a purity of 95%.

Paraldehydum (B.P.). Sp. gr., 0.998 to 1.000. Distillation range, not more than 10% below 123° , the remainder between 123° and 126° . M.p., not below 11° . Complies with limit tests for acidity, acetaldehyde and peroxidised compounds. Paraldehydum, *U.S.P. XII*, should comply with purity tests for impurities derived from fusel oil, amyl alcohol, sulphate, chloride, free acid and acetaldehyde.

The analytical examination of paraldehyde is described, including a procedure for its determination in cases of paraldehyde poisoning.—D. J. T. Bagnall, A. Smith and A. R. Tankard, *Analyst*, 1939, 857.

GELATINUM

Gelatinum (B.P.). A 2% *w/v* hot aqueous solution is odourless and sets to a transparent or translucent jelly on cooling. Sulphur dioxide limit, 1000 parts per million. Ash, not more than 2%. The *U.S.P. XII* sulphur dioxide limit for Gelatinum is 0.004%, by distillation with phosphoric acid into N/10 iodine and precipitation with barium chloride. The *P. Helv. V* allows 0.05% SO_2 in Gelatina animalis.

A British Standard Specification (B.S.S. No. 757—1937), has been issued by the British Standards Institution describing methods for testing gelatins. The specification describes both physical tests, which are mainly determined by the degree of hydrolysis to which the raw material was subjected, and chemical tests, which are concerned with freedom from associated substances. The former include determinations of the jelly strength, viscosity, melting-point, foam, water absorption, solubility, keeping quality, colour and clarity, pH. Chemical determinations described include moisture, grease, ash, sulphur dioxide, chlorides, arsenic and heavy metals.

GELSEMIUM

Gelsemium (B.P.C.). Contains not more than 2% of foreign organic matter. The *N.F. VII* allows the same limit of foreign matter; acid-insoluble ash limit, 2%.

A standard of 0.5% total alkaloids for the root, and 0.05% for the tincture has been suggested.

For a lengthy account of the work of L. E. Sayre, C. W. Moore and others (1911) on the gelsemium alkaloids, also the suggestion to use the name *sempervirine* to replace the amorphous gelsemine, see *Yearb. Pharm.*, 1920, 10.

Identification of Gelsemium. It does not contain aesculin. The fluorescent body is scopoletin (aesculetin-5-methyl ether). If 0.5 g. of the ground drug be heated in a tube with chloroform, the mixture filtered and the filtrate shaken with water to which a few drops of dilute ammonia have been added, the aqueous layer on separation shows a distinct blue fluorescence, indicating presence of scopoletin.—F. Tutin, *Pharm. J.*, i/1912, 157.

The alkaloidal substances in gelsemium root are, according to L. E. Sayre (*Drugg. Circ.*, 1919, 63, 423), the crystalline alkaloids gelsemine and sempervirine, the former being white and the latter reddish-brown, and also the two amorphous alkaloidal substances gelsemidine and gelsemoidine. Gelseminine is the name given often to a mixture of the amorphous bases, which are stated to be mainly responsible for the physiological action of gelsemium. The root also contains non-alkaloidal colour substances.

Assay. Introduce 10 g. of the root and 5 g. of acid-washed sand, both in No. 60 powder, into a pear-shaped separating funnel of about 300 ml. capacity, and add 100 ml. of a mixture of three volumes of ether and one volume of chloroform, shake well and set aside for ten minutes; add 5 ml. of dilute solution of ammonia B.P., and shake for one minute at ten-minute intervals during one hour. Insert a plug of cotton wool into the stem of the funnel and allow the liquid to percolate into another separating funnel. When the liquid ceases to flow, pack the drug firmly and continue the percolation with further quantities of the solvent until complete extraction of the alkaloids is effected. Test for complete extraction by evaporating 2 ml. of percolate, collected separately in a dish, dissolving the residue in a few drops of 0.1 N sulphuric acid and adding one drop of 0.1 N iodine. No precipitate or turbidity should form. To the percolate add 30 ml. of N sulphuric acid, shake well, allow to separate and run off the lower layer. Continue the extraction of the alkaloids with 10 ml. portions of 0.1 N sulphuric acid until extraction is complete. Wash the mixed acid solutions with about 10 ml. of chloroform and run off the latter into a second separating funnel containing 20 ml. of 0.1 N sulphuric acid, shake, allow to separate and reject the chloroform. Repeat the washing of the liquid in the

first separating funnel with two further quantities of 5 ml. each of chloroform, transfer each in turn to the second separating funnel, wash with the same aqueous acid liquid, allow to separate and reject the chloroform as before. Transfer the acid liquid from the second to the first separating funnel, make just alkaline with dilute solution of ammonia B.P. and add 2 ml. in excess; shake with successive portions of chloroform until complete extraction of the alkaloids is effected, washing each chloroform extract with the same 20 ml. of water contained in another separating funnel. Distil off the chloroform, add 2 ml. of absolute alcohol to the residue, evaporate and dry at 60° for thirty minutes. Dissolve the residue in 2 ml. of alcohol 95%, warming until dissolved, add 2 ml. of 0.1 N sulphuric acid and 10 ml. of water, cool and titrate with 0.1 N sodium hydroxide from a microburette, using methyl red as indicator. Each ml. of 0.1 N acid is equivalent to 0.0366 g. of the alkaloids of gelsemium calculated as gelsemine.—Poisons Sub-Committee of the Analytical Methods Committee, *Analyst*, 1941, 108.

Biological Assay. A method using pigeons is recommended and fully described.—B. V. Christensen and L. G. Gramling, *J. Amer. pharm. Ass.*, 1938, 1208.

GENTIANA

Gentiana (B.P.). Contains not more than 2% of foreign organic matter. Water-soluble extractive, not less than 33%. Ash, not more than 6%. Gentiana, *U.S.P. XII*, should yield not less than 30% of water-soluble extractive; moisture, not more than 15%; limit of foreign matter, 2%.

Calumba (B.P. Add. I). Foreign organic matter, not more than 2%. Ash, not more than 9%. Alcohol (60%) soluble extractive, not less than 12%. Calumba, *N.F. VII*, should contain not more than 1% of foreign matter and yield not more than 2.5% of acid-insoluble ash.

GLYCERINUM

Glycerinum (B.P.). $C_3H_8O_3 = 92.06$. Sp. gr., 1.260 to 1.265 (corresponding to from 98 to 100% of $C_3H_8O_3$). $n_{D_{20}^0}$, 1.470 to 1.473. Ash, not more than 0.01%. Glycerinum, *U.S.P. XII*, contains not less than 95% of $C_3H_8O_3$; sp. gr. at 25°, not below 1.249; 5 ml. complies with a test for carbonisable substances.

Glycérine Officinal, *Fr. Cx.* 1937, contains about 3% of water; sp. gr. at 20°, not less than 1.255. Glycerinum, *P.G. VI*, contains from 84 to 87% of anhydrous glycerin; sp. gr., 1.221 to 1.231. Glycerinum, *P. Helv. V*, has a sp. gr. of 1.224 to 1.234 and contains from 84 to 88% of $C_3H_5(OH)_3$, whilst Glycerinum concentratum has a sp. gr. of 1.260 to 1.266 and contains at least 98% of $C_3H_5(OH)_3$.

Detection of Glycerin. By oxidation with permanganate and treatment with Schiff's reagent, glycerin gives a positive coloration in 0.04% solution. The common organic acids do not interfere.—*J. chem. Soc. Abstr.*, ii/1925, 162. See also *Analyst*, 1926, 382.

The identification of glycerin by a bacterial method.—A. Castellani and F. E. Taylor, *J. trop. Med. (Hyg.)*, Oct. 15, 1924, 271.

The following test is based on oxidation to dihydroxyacetone and conversion of the latter to pyrotartaric aldehyde. 2 drops of the solution to be tested is placed in a test-tube which is then filled with bromine vapour, covered, and

heated for ten minutes at 85° to 90° . The cover is removed, heating is continued for 15 minutes, and a crystal of sodium sulphite is added to remove all traces of bromine. 2 to 3 ml. of sulphuric acid is added carefully, with simultaneous cooling, followed by a little solid metahydroxybenzoic acid and the mixture is heated for 15 minutes at 65° to 70° . A green fluorescence shows the presence of glycerin. The sensitivity is 5 γ in 0.05 ml. of solution. The reaction can be observed in presence of six times the amount of glycol or glucose, or twice the quantity of sucrose, but levulose causes more interference.—E. Eegriwe, *Z. anal. Chem.*, 1935, 31.

Determination. Reflux a mixture of 10 ml. approximately 0.025 M glycerin solution, 50 ml. N/10 ceric sulphate and 50 ml. 2N sulphuric acid for one hour. Cool, add 1 ml. of 0.01% aqueous solution of xylene-cyanol FF and titrate the excess of ceric sulphate with N/10 ferrous ammonium sulphate. This method gives high results if the glycerin concentration is less than 1 g. per litre. Sodium hypobromite has also been used in estimating glycerin. Quantitative oxidation to carbon dioxide and water is obtained when 15–30 ml. N/10 sodium hypobromite solution containing not less than 8 g. per litre of sodium hydroxide is used, in excess. The mixture is boiled for 90 minutes and the excess hypobromite assayed iodometrically.—B. Cuthill and C. Atkins, *J. Soc. chem. Ind., Lond.*, 1938, 89.

Determination by Means of Periodates. An excess of solid potassium periodate is added to the neutral solution, the mixture is shaken for 20 minutes and the formic acid titrated with alkali hydroxide using methyl red. The method is applicable in the presence of substances which do not react with periodic acid in the cold or of substances which react but do not give acids or alkalis.—M. A. Malaprade, *Bull. Soc. chim. Fr.*, 1937, 906.

Garrratt (*Drugs and Galenicals*) recommends the method of Shukoff and Schestakoff for determination of glycerin in resorcin ointment:—To a solution with slight excess of sulphuric acid and filtered, add a slight excess of potassium hydroxide solution and evaporate not above 80° to a syrup. Mix with 20 g. of anhydrous sodium sulphate and extract with acetone for 4 hours in a thimble and Soxhlet; evaporate, and dry at 75° to 80° .

Ethylene Glycol: Detection in the Presence of Glycerin. To 10 ml. of test sample add 2 ml. of nitric acid and evaporate to 1 ml., then add 5 ml. of 20% w/v ammonium chloride and allow to cool. Make alkaline by adding, 1 ml. at a time, 10% sodium hydroxide until there is a slight smell of ammonia. Boil off the free ammonia and add 1 ml. of 10% w/v barium chloride. After cooling the mixture for about 15 minutes, filter and wash with about 5 ml. of water. Rinse the precipitate into a boiling tube with 5 ml. of water, add 7.5 ml. of 2N sulphuric acid and heat to boiling. Cool and filter. The filtrate is treated with 0.6 N permanganate until no more is decolorised in the cold and is then boiled. Any further decolorisation shows the presence of oxalic acid, that is of glycol, in the original product. The test will detect 1% of glycol in water or 3% in a mixture of 70% of glycerin and 27% of water.—A. W. Middleton, *Analyst*, 1934, 522.

Before applying the above test triethanolamine must be removed and the following method is suggested. To the sample containing triethanolamine and ethylene glycol add 8 ml. of water, 2 ml. of 50% sulphuric acid and 5 ml. of 30% solution of phosphotungstic acid. Allow to stand in a refrigerator for 3 days, filter, evaporate to small bulk with excess of barium carbonate, extract with ethyl alcohol and evaporate the alcohol.—C. J. Eastland, N. Evers and T. F. West, *Analyst*, 1937, 266.

Determination of Ethylene Glycol. Mix 50 ml. 0.1 N potassium permanganate, 10 ml. 0.025 M glycol solution and 30 ml. 4N sodium hydroxide; stand 1½ hours, add 50 ml. 4N sulphuric acid and stand for a further hour. Finally, add 10 ml. 10% potassium iodide and titrate liberated iodine with thiosulphate. Repeat omitting glycol.—R. Cuthill and C. Atkins, *Analyst*, 1938, 259.

GLYCYRRHIZA

Glycyrrhiza (B.P.). Water-soluble extractive, not less than 20%. Ash limits: peeled drug, not more than 6%; unpeeled drug, not more than 10%. Acid-insoluble ash, not more than 2.5%. Powdered liquorice B.P. is the powder of the peeled drug;

a powder of the unpeeled drug is not used except when expressly named. Glycyrrhiza, *U.S.P. XII*, yields not more than 2.5% of acid-insoluble ash.

Fr. Cx. 1937. Determination of glycyrrhizin in Suc de Régliſſe. Dissolve 3 g. dried at 100° in 30 ml. of water with 5 drops of ammonia solution and filter; to 20 ml. of filtrate (2 g. dried drug) add 2.5 ml. of sulphuric acid and after 24 hours filter, and wash the residue with 30 ml. water; dissolve the residue in 2 ml. ammonia solution, filtering through the same filter and washing with water made ammoniacal (5 drops to 10 ml.). Evaporate and dry at 100° for one hour, weigh and add correction for the glycyrrhizin dissolved in mother liquor and washings (0.04 g. for 40 ml. total). The glycyrrhizin ammonia should be not less than 10%.

The name "glycyrrhizin" applies to the sweet substance found in liquorice root, a mixture of calcium and potassium glycyrrhizates.

Tschirch discovered that glycyrrhizic acid is the diglycuronic acid ester of glycyrrhetic acid. It has glycosidal properties. Glycuronic acid is of importance in animal life—an unexpected fact, since the most varied sugars are at the disposal of a plant if it wishes to form glycosides.

A minimum of 9% of "glycyrrhizin" should be present in normally prepared edible juices—they should not contain more than 18% of sugars, reducing and non-reducing. In order to determine whether the starch be actual or added, the sample should be powdered, extracted with water and the residue taken up with 3% ammonia solution. The insoluble matter should never exceed 6%. Examine this under the microscope to trace source of starch, i.e., whether added or of the same character as that in the root. The amount not dissolved in 70% alcohol should not exceed 16.5%. Gum should never be present in pure liquorice juice.—Parry, *Chem. & Drugg.*, i/1911, 183.

GOSSYPIUM

Gossypium Absorbens (*B.P.C.*). Absorbent cotton wool. A thin layer, equivalent to about 0.5 g. for an area of 70 sq. in., when, viewed between glass plates, by transmitted light, is not more neppy than the standard sample kept at the Manchester Testing House. 1 g. compressed to about 20 ml. and placed lightly on water at 20° sinks or becomes saturated within 10 seconds. Average length of staple, not less than $\frac{1}{8}$ inch. Water-soluble extractive, not more than 0.5%. Ash, not more than 0.5%.

The greasy material on absorbent cotton wool consists mainly of fatty acids with a fairly large proportion of unsaponifiable matter. In commercial samples this substance is present in sufficient quantity to cover the fibres with a film of molecular thickness and the absorbency will be greater or less according to whether the fatty acids are oriented with the carboxyl groups outwards or inwards. In the latter case wetting is due to rupture of the film consequent upon the swelling of the fibres under the influence of water vapour diffusing through the grease, and is a slower process. The orientation of carboxyl groups in the grease can be affected by external conditions, but samples impregnated with purely paraffinoid substances show only the second type of wetting.—R. Maxwell Savage, *J. Soc. chem. Ind., Lond.*, 1934, 379.

Surgical Dressings. The *B.P.C. Supp. IV* directs that all weights and counts of yarn refer to those obtained under standard humidity conditions, the materials being exposed to an atmosphere of 65 to 70% relative humidity for a period of at least 6 hours.

Prescribed Methods.

To avoid error due to comparatively large spaces between threads in fabrics of open texture, a length of ten inches shall be counted for number of threads per inch.

Elasticity is determined by applying to the movable end of crêpe and adhesive bandages (the other end being held in a fixed grip) by means of a spring dynamometer, a tension of 6 pounds per inch width. The tension is maintained for 1 minute and the stretched length measured; the bandage is then released immediately and measured after 5 minutes, the length being expressed as percentage of the stretched length.

Water-soluble Extractive is determined by the loss in weight after well washing the dried sample from the moisture determination, followed by hand wringing 12 successive times, any loose threads, which should be collected by passing the washing through a fine sieve, being added. The residue is dried at 100° and weighed. For wool and union fabrics the wash water should be at 45° to 50°.

Foreign Matter. By continuous extraction with a solvent, such as chloroform, ether, carbon tetrachloride, of the dried sample (wool should be further extracted with alcohol); after airing off the solvent, the cloth is washed as in the water-soluble extractive determination. If starch is present, it is soaked in 0.5% malt extract solution at 50° and then heated to 70° for 15 minutes, followed by washing with hot running water 12 times as before. The residue, with any collected loose threads or fibres is dried at 100°, and weighed. In wool and grey cotton an allowance of 3% is made for loss of natural impurities. The cleaned sample is ashed to indicate that all insoluble matter is removed and 0.2% allowed for the natural ash.

Cotton and Wool. The dried material after extraction of the foreign matter is boiled in 5% sodium hydroxide solution for 10 minutes, removed, the treatment repeated and then well washed. The residue plus loose threads and fibres, collected as before, is dried at 100° and weighed as dry cotton. For unbleached cotton an allowance for loss of natural constituents of 4% is made. The loss in weight is that of dry wool. Standard moisture regains of 8.5 and 16% respectively are added.

Tensile Strength. Six specimens of the material of the specified width and measuring 7 inches held between the jaws of a Goodbrand machine having a constant rate of traverse of 18 inches per minute, are submitted to test in the machine after exposure to an atmosphere of 65 to 70% relative humidity for at least 6 hours. The average of the minimum breaking load for the 6 specimens is the tensile strength.

Carbasus Absorbens (B.P.C.). Absorbent gauze. Water-soluble extractive, not more than 0.5%. Weight per sq. yd., not less than 180 gr. Average number of threads per inch, not less than 19 in the warp and 15 in the weft. A test for absorbency is included.

Carbasus Absorbens in Tænia (B.P.C.). Complies with the same standard as Carbasus Absorbens for material and water-soluble extractive. Weight of a piece 2 inches × 6 yards, not less than 190 grains. Average number of threads per inch, not less than 30 in the warp and not less than 25 in the weft. A test for absorbency is included.

Cellulosum Ligni (B.P.C.). Cellulose wadding. Superficial area, not less than 1500 sq. in. per pound. Moisture limit, 10%. Ash limit, 0.5%. Chloroform extractive, not more than 1%. 1.5 g. compressed to about 20 ml. and placed on water at 20° sinks or becomes saturated within 5 seconds.

Curatio Normalis III (B.P.C. Supp. II). The unstretched spread fabric, after removal of the medicated pad, weighs not less than 4.4 gr. per sq. inch and after the further removal of the rubber adhesive compound and other foreign organic matter, not less than 2.2 gr. per sq. inch (correction being made for natural moisture regain). The fabric should be of two-fold crêpe yarn,

having not less than 44 turns one way and standard two-fold yarn the other way, both being of good uniform grade. Threads per sq. inch, not less than 70, when fully stretched in the elastic direction, and on immediate release returns to a length not more than 80% of the fully stretched length.

Curatio Normalis IV (*B.P.C. Supp. II*). Complies with the same standard for the elastic cotton fabric described under Curatio Normalis III.

Curatio Normalis V (*B.P.C. Supp. II*). The elastic cotton fabric complies with the same standard.

Curatio Normalis VI (*B.P.C. Supp. II*). Complies with the standard for elastic cotton fabric described above.

Linteum Absorbens (*B.P.C.*). Absorbent lint. Water-soluble extractive, not more than 0.5%. Superficial area, 230 to 250 sq. in. per ounce. Minimum average number of threads per inch, 39 in the warp and 24 in the weft. A piece, 3 × 3 in., placed unraised side downwards on water at 20° becomes saturated in 10 seconds.

Tela Carbasi et Gossypii (*B.P.C.*). Gauze and cotton tissue. Superficial area, not less than 1800 sq. in. per pound. The absorbent gauze and absorbent cotton wool comply with the standards for Carbasus Absorbens and Gossypium Absorbens respectively.

Tela Carbasi et Gossypii Capsici (*B.P.C.*). Capsicum tissue. The absorbent gauze should comply with the standard for Carbasus Absorbens, with the exception of the colour and the weft, which has not less than 12 threads per inch. Superficial area not less than 1800 sq. in. per pound.

Tela Carbasi et Ligni (*B.P.C.*). Cellulose tissue. The absorbent gauze should comply with the standard for Carbasus Absorbens, with the exception that the weft has not fewer than 12 threads per inch. The cellulose wadding complies with the *B.P.C.* Cellulosum Ligni standard. Superficial area, not less than 1350 sq. in. per pound.

A summary of the circular C.423 issued by the National Bureau of Standards, U.S. Dept. of Commerce, containing a compilation of methods for the microscopic identification of commercial fibres including paper, jute, cotton, flax rays, silk and wool, is given in *Analyst*, 1939, 425.

Protectives

Fabric of Battiste and Jaconet. 3-inch squares of the material are immersed in a solvent (benzene, toluene, xylene, etc.), heated to boiling and kept boiling if necessary. The cuttings are removed, the swollen proofing removed and the fabric heated in liquid paraffin at a temperature not exceeding 150°. The fabric is shaken and extracted with petroleum ether, any filling removed as for determination of foreign matter, and finally dried at 100°. To the weight allowance for natural moisture regain is made.

Battista (*B.P.C.*). With boiling water, or when heated with steam, battista must show no stickiness or deterioration. Weight

per square yard not less than 5 oz., of the fabric alone not less than 2 oz., and the difference of these not less than 3 oz. Average threads per inch, not less than 104 in the warp and 72 in the weft.

The following alternative specification for Battista *B.P.C.* has been published (see *Pharm. J.*, i/1943, 130). Weight per square yard not less than 5.75 oz.; weight per square yard of the fabric alone not less than 2 oz.; the difference between the weight of the fabric per square yard and the proofed material is not less than 3.75 oz. Average threads per inch, not less than 104 in the warp and 72 in the weft. Tensile strength per 1 inch strip, not less than 40 pounds in the warp and not less than 25 pounds in the weft. The proofing contains not more than 35% of mineral matter.

Emplastrum Adhesivum (*B.P.C. Supp. IV*). Weight per sq. yard, not less than $7\frac{1}{4}$ ounces. Tensile strength of the warp per inch width, not less than 45 pounds. Weight of base cloth, after removal of adhesive, etc., and corrected for moisture regain, not less than $3\frac{3}{4}$ ounces per sq. yard. Weight of adhesive, etc., not less than $3\frac{1}{2}$ ounces per sq. yard. A specification of the quality of the cloth is included. Average number of threads per inch, not less than 72 in the warp, not less than 70 in the weft.

Jaconettum (*B.P.C.*). With boiling water or when heated with steam jaconet must show no stickiness or deterioration. Weights per square yard not less than 6 oz. total, fabric alone 2 oz., and the difference 4 oz. Not less than average number of 104 threads in the warp and 72 threads in the weft per inch. An alternative specification has also been prescribed for Jaconettum *B.P.C.* (see *Pharm. J.*, i/1943, 130).

Sericum Oleatum (*B.P.C.*). Oiled silk. Minimum weights per square yard for oiled fabric 2.5 oz., and for silk fabric alone 0.33 oz. Average number of threads per inch, not less than 120 in the warp and 85 in the weft.

Sericum Oleatum Factitium (*B.P.C.*). A specification has been published for oiled artificial silk for use in place of oiled silk (see *Pharm. J.*, i/1943, 45). Weight per square yard of waterproof fabric, 2.25 to 2.75 oz.; weight of the fabric alone and after allowance for natural moisture regain, not more than 0.85 oz. per square yard. Average number of threads per inch, not less than 100 in the warp and 100 in the weft.

Sindon Oleata (*B.P.C.*). Oiled cambric. Minimum weights per square yard for the oiled fabric 4 oz., for cotton fabric alone 1.5 oz., and the difference 2.5 oz. Average number of threads per inch, not less than 74 in the warp and 68 in the weft.

HEPAR

Extractum Hepatis Siccum (*B.P.*). Should contain not less than one-tenth its weight of sodium chloride and should be

packed in tubes, each containing the equivalent of 225 g. of original liver. *Extractum Hepatis, U.S.P. XII*, complies with the specifications of the U.S.P. Anti-anæmia Preparations Advisory Board, by which satisfactory clinical data regarding treatment of Addisonian pernicious anæmia and evidence that the satisfactory result is produced by the dose given shall be available; the preparation must be labelled with the average dose.

Extractum Hepatis Liquidum (B.P.). 1000 ml. should contain the equivalent of 8000 g. of original liver, not less than the equivalent of 10% *v/v* of 95% alcohol, and not less than 20% *v/v* of glycerin. *Liquor Hepatis, U.S.P. XII*, conforms to specifications of the U.S.P. Anti-anæmia Preparations Advisory Board.

Liver extracts contain chromogenic purine constituents giving a blue colour with urea cyanide reagent followed by Folin's uric acid reagent. Part of the chromogen is precipitable by lead acetate and this fraction was found absent from the liver of a patient dying from untreated pernicious anæmia, but present in that of a patient successfully treated for pernicious anæmia but dying from other causes. The chromogenic purine specifically cures canine black tongue and since both this disease and pellagra are characterised by spinal cord degeneration, which is also associated with pernicious anæmia and about which there have been conflicting reports as to the efficacy of liver extract, it may well be that liver extracts effective in cord degeneration are rich in purine. Some highly potent liver extracts for parenteral injection have very little chromogen activity.—G. E. Shaw, *Quart. J. Pharm.*, 1937, 380.

A reticulocyte response is obtainable in pigeons even with liver extracts which are clinically inactive.—G. E. Shaw and J. Pritchard, *Quart. J. Pharm.*, 1937, 517.

Assay. There is no satisfactory biological test for liver extract. The pigeon method introduced by Vaughan and her colleagues has been investigated by Gurd, *Quart. J. Pharm.*, 1935, 39, who found that when sufficient care is taken to stain the red cells of the pigeon, it becomes impossible to make a sharp distinction between those which have a reticulum and those which have not. If an arbitrary distinction is made of those with a heavy reticulum from those with a lighter one, then no effect on the number of reticulocytes is produced when liver extract is injected.

A method of assay has been developed, using a test upon guinea-pig bone marrow preparations *in vitro* which apparently makes possible the quantitative estimation of anti-anæmic potency of liver concentrates. The method depends upon measurement of the migration of cells from isolated living bone marrow embedded in a coagulum of plasma to which various concentrations of liver extract have been added. At certain optimal concentrations of liver extract the migration is increased and the concentration thus effective is an index of the potency of the liver extract. A curve has been constructed showing the relationship between the dilution of liver concentrates at which maximum migration of cells from bone marrow occurred, and the *U.S.P.* potency of these concentrates. It would appear that this curve may be used for determination of the expected clinical potency of unknown liver extracts.—N. Pace and R. S. Fisher, *J. Pharmacol.*, 1942, 74, 256.

Ventriculus Desiccatus. The following upper limits are suggested:—Ash 5%, calculated on a fat- and moisture-free basis; fat 15% if not defatted, or 5% if the fat is extracted; moisture 5%. It is also desirable that the preparation should be free from organisms giving acid and gas in MacConkey's medium.—K. Bullock, *Quart. J. Pharm.*, 1936, 381.

Assay by Casein Digestion. A valuable guide to the clinical activity of desiccated stomach preparations is provided by their peptic activity and the following method has proved of value for the determination of the enzyme activity of stomach preparations at various stages of manufacture. 0.5 g. of pure casein is dissolved in 40 ml. of water and 20 ml. of N/1 sodium hydroxide by warming on a water-bath. To the resulting solution is added 30 ml. of N/10 hydrochloric acid. The liquid is filtered and the filtrate made up to 100 ml. A weighed quantity of the stomach preparation to be tested, which has been passed through a No. 40 sieve, is shaken for 2 hours with dilute hydrochloric

acid (120 ml. of N/1 acid with water to 1 litre) and filtered. 1 g. of the preparation in 1 litre of acid is usually suitable. To carry out the test, into each of seven test-tubes is placed 1 ml. of pepsin solution followed by 0.7 ml. 0.5 ml. . . . 0.1 ml. of the casein solution. The contents of each tube are diluted to 4 ml. with the diluted hydrochloric acid and are then incubated at 40° for one hour. After incubation 0.5 ml. of saturated ammonium sulphate solution is added to each tube and their turbidities compared as rapidly as possible. The comparison is made by holding the tubes in front of a printed card, the print being a light face Gill Sans type, 8-point size. The tube through which the print is just readable is known as the index tube. The amount of the hog stomach preparation in this tube is that required to digest 0.005 g. of casein and from this the weight of casein digested by 1 g. of preparation may be calculated.—E. E. Rymill and C. A. Macdonald, *Quart. J. Pharm.*, 1937, 323.

Heparin

Assay. The anticoagulant potency of heparin is tested against either freshly drawn blood or various artificial clotting systems which can be clotted when desired. Three methods of assay are described:—(1) The Howell assay as modified by Charles and Scott, in which the degree of clotting in samples of cat's blood containing varying amounts of the unknown is compared with similar samples containing a standard preparation, (2) a new thrombin assay, in which the unknown and standard heparin solutions are titrated against a system consisting of oxalated beef blood and thrombin, using a constant clotting time, and (3) the Fischer assay, in which the clotting time of chicken plasma on addition of thrombokinase is determined for varying concentrations of unknown and standard heparin. Factors which affect the results of the assays are discussed. The crystalline heparins isolated from different species show marked differences in their specific biological activity. It has been found that their relative anticoagulant activities vary with the method of assay.—L. B. Jaques and A. F. Charles, *Quart. J. Pharm.*, 1941, 1.

A simple method of comparing the activity of anti-coagulants is described. Fresh rabbits' blood is added to two series of test-tubes, one containing definite amounts of the anti-coagulant under test, the other definite amounts of a standard solution of the sodium salt of heparin. By comparing the tubes, the activity of the anti-coagulant can be found in terms of the standard preparation. The error is stated not to exceed 10%.—F. Schütz, *Quart. J. Pharm.*, 1941, 45.

HEXAMINA

Hexamina (B.P.). $C_6H_{12}N_4 = 140.1$. Contains not less than 99% of $C_6H_{12}N_4$. Ash, not more than 0.05%. Assayed by boiling with N/1 sulphuric acid until all the formaldehyde produced has been evolved, and back titrating the excess acid with N/1 sodium hydroxide to methyl orange. By the same method Methenamina, *U.S.P. XII*, should contain 99% of the pure substance, after drying over sulphuric acid for four hours.

Tabellæ Methenaminæ et Sodii Biphosphatis (N.F. VII). Contain 92.5 to 107.5% of the labelled amount of methenamine, including all tolerances. Limit tests for aluminium, calcium and ammonium salts are included. Assayed by boiling with dilute sulphuric to remove formaldehyde, subsequent ammonia distillation into N/2 sulphuric acid, and titration with N/2 sodium hydroxide using methyl red indicator.

A tentative quantitative method for the determination of hexamine in tablets is described in *Methods of Analysis (A.O.A.C., 1940, 576)*.

A method for the quantitative determination of hexamine using calcium hypobromite.—E. F. Slowick and R. S. Kelley, *J. Amer. pharm. Ass., Sci. Edn.*, 1942, 15.

HYDRARGYRUM

Hydrargyri Cyanidum (B.P.C. Supp. IV). $C_2N_2Hg = 252.6$. Contains not less than 99% of $Hg(CN)_2$. Residue on ignition, not

more than 0.1%. Limit tests for chloride and mercuric oxycyanide are described. Assayed by addition of sodium chloride and potassium iodide, and titration with N/10 hydrochloric acid to methyl orange.

Carbasus Hydrargyri et Zinci Cyanidi (B.P.C.). Double cyanide gauze contains mercury equivalent to from 0.5 to 1.5% of $\text{Hg}(\text{CN})_2$, and zinc equivalent to from 1.5 to 3% of $\text{Zn}(\text{CN})_2$. A nitric acid extract of the gauze and washings is adjusted to volume; zinc is determined in one portion by the B.P. method for Zinci Sulphas, by precipitation with mercuric ammonium thiocyanate solution and subsequent titration with potassium iodate; mercury is precipitated as sulphide from the remaining portion after neutralisation and just acidifying with hydrochloric acid.

Hydrargyri Iodidum Flavum (B.P.C.). $\text{HgI} = 327.5$. Assayed by interaction with N/10 iodine and potassium iodide, followed by back titration with N/10 sodium thiosulphate; a purity of the dried substance of not less than 99% should be indicated. Loses not more than 0.5% on drying over sulphuric acid, and leaves not more than 0.2% of residue on volatilisation. The N.F. VII specifies that the substance dried over sulphuric acid shall contain 99% of HgI ; residue on volatilisation, not more than 0.1%.

Tabellæ Hydrargyri Iodidi Flavi (N.F. VII). Contain 91 to 109% of the labelled amount of yellow mercurous iodide, including all tolerances. Assayed by dissolving in water and hydrochloric acid and titrating with M/50 potassium iodate using chloroform as indicator.

A tentative quantitative method for the determination of mercurous iodide in tablets is described in *Methods of Analysis (A.O.A.C., 1940, 621)*.

Hydrargyri Iodidum Rubrum (B.P.). $\text{HgI}_2 = 454.5$. After standing for 10 minutes with zinc powder and water and filtering off the amalgam formed, the filtrate and washings are treated with excess N/10 silver nitrate and nitric acid and titrated with N/10 ammonium thiocyanate; not less than 99% of the pure salt should be indicated. Leaves not more than 0.1% of residue on volatilisation. The N.F. VII assays by titration, in the presence of hydrochloric acid, with M/20 potassium iodate using chloroform as indicator, and requires the substance dried over sulphuric acid to contain not less than 99% of HgI_2 .

Tabellæ Hydrargyri Iodidi Rubri (N.F. VII). Contain 91 to 109% of the labelled amount of red mercuric iodide, including all tolerances. Assayed by dissolving in water and hydrochloric acid and titrating with M/50 potassium iodate, using chloroform as indicator.

Solvellæ Hydrargyri Iodidi (B.P.C.). Contain 8.75 grains of HgI_2 , and 1 tablet in a pint of water gives a 1 in 1000 solution of HgI_2 . Some makers prepare them to contain $8\frac{3}{4}$ grains of anhydrous mercuric potassium iodide ($\text{HgI}_2 \cdot \text{KI}$) with a sufficiency of potassium iodide in excess to make the body $\text{HgI}_2 \cdot 2\text{KI}$. One dissolved in 1 pint of water makes a 1 in 1000 solution of mercuric potassium iodide and each contains 6.4 grains of HgI_2 . A trade custom has developed, however, of making them on various

assumptions, e.g., to contain $8\frac{1}{2}$ grains of the soluble iodide, $\text{HgI}_2 \cdot 2\text{KI}$, which renders the content of mercuric iodide in the solution far less, namely, 5 grains.

To estimate the mercury in tablets of this kind, formalin reduction as recommended by E. Rupp is used. For the iodine, the iodate reaction in presence of strong hydrochloric acid is suitable. $2\text{HI} + \text{HIO}_3 + 3\text{HCl} = 3\text{I}_2 + 3\text{H}_2\text{O}$. The whole matter is well dealt with in a paper by A. J. Jones, *Chem. & Drugg.*, i/1920, 523.

Garratt (*Drugs and Galenicals*) recommends the method of Sage and Stevens (*Chem. & Drugg.*, i/1932, 84). Reflux with 2 g. of zinc filings in acetic acid (50%) for 30 minutes, filter, wash, and dissolve the amalgam in nitric acid; oxidise with permanganate and titrate with N/10 potassium thiocyanate. The iodine may be determined in the acetic acid solution and washings by titration with M/20 potassium iodate in presence of hydrochloric acid.

Hydrargyri Iodidum Viride (B.P.C. Supp. IV). Contains not less than 24.5% of I, determined by interaction with zinc dust and small quantity of water and acetic acid, dilution, filtration, and washing of the insoluble amalgam, followed by titration of the soluble iodide formed, with excess N/10 silver nitrate and N/10 ammonium thiocyanate using ferric ammonium sulphate indicator; Hg content not more than 75%, as shown by solution of the amalgam from the iodine assay in dilute nitric acid, oxidation with potassium permanganate solution and titration with N/10 ammonium thiocyanate.

Hydrargyri Oxidum Flavum (B.P.). $\text{HgO} = 216.6$. Loses not more than 1% when heated at 150° for 1 hour, and then contains not less than 99.3% of the pure substance. Residue on ignition, not more than 0.5%. Assayed by titration in nitric acid solution with N/10 ammonium thiocyanate. The *U.S.P. XII* requires the substance dried to constant weight at 110° to contain 99.5% of HgO .

The temperature of 150° , specified in the *B.P.* as that at which the substance is to be dried for calculating the percentage strength, is too high since marked amounts of mercurous oxide are produced at this temperature. This decomposition and that of the mercurous oxide occurring as an impurity can be minimised by drying at 70° , at which temperature no decomposition takes place.—G. J. W. Ferrey, *Quart. J. Pharm.*, 1933, 406.

Hydrargyri Oxidum Rubrum (B.P.C.). $\text{HgO} = 216.6$. Loses at 150° not more than 1%, and is then of 99.3% purity. Residue on volatilisation, not more than 0.3%. The *N.F. VII* requires the salt dried at 120° to contain not less than 99.5% of HgO .

Hydrargyri Oxycyanidum (B.P. Add. I). $\text{HgO} \cdot 3\text{Hg}(\text{CN})_2 = 974.5$. Contains 20 to 22% of HgO and 77 to 79% of $\text{Hg}(\text{CN})_2$. Loss at 100° , not more than 1%. Residue on ignition, not more than 0.1%. Assayed for mercuric oxide by treatment with sodium chloride and titration with N/10 hydrochloric acid to methyl orange; and for mercuric cyanide by continuing the titration after adding potassium iodide. 1 g. produces a clear solution with 200 ml. of water.

Oxycyanure Mercurique, *Fr. Cx.* 1937, assayed similarly contains 14.4 to 16.35% of HgO and 83.75 to 84.63% of mercuric cyanide. *Hydrargyrum oxycyanatum*, *P.G. VI*, contains

33.3 to 35.2% of $\text{Hg}(\text{CN})_2 \cdot \text{HgO}$, corresponding to 15.37 to 16.25% of HgO and 84.6 to 83.8% of $\text{Hg}(\text{CN})_2$. Hydrargyrum oxycyanatum, *P. Helv. V*, contains from 15.37 to 16.25% of HgO and from 83.75 to 84.63% of total $\text{Hg}(\text{CN})_2$.

Hydrargyri Perchloridum (B.P.). $\text{HgCl}_2 = 271.5$. Contains not less than 99.5% of the pure salt. Residue on volatilisation, not more than 0.1%. Assayed by reduction in presence of potassium iodide with formaldehyde and sodium hydroxide solutions, acidifying with acetic acid and adding excess N/10 iodine and, when the precipitate is completely dissolved, back titrating with N/10 sodium thiosulphate. Hydrargyri Bichloridum, *U.S.P. XII*, after drying for 18 hours over sulphuric acid should contain not less than 99.5% of HgCl_2 . Assayed by precipitation as sulphide, washing and drying at 110° .

In the *B.P.* assay process the addition of 5 ml. of a mixture of 2 vols. of ether and 1 vol. of chloroform enables rapid solution of the precipitated mercury to be effected.—H. Brindle, *Quart. J. Pharm.*, 1932, 432.

Modification of Rupp's Method. The addition of gelatin retains the reduced mercury in a finely divided condition and avoids trouble due to the metal failing to react with iodine owing to aggregation. The mercuric solution is treated with sufficient potassium iodide (about 1 g.) to form a double salt, rendered alkaline with 5N sodium hydroxide solution (an excess of 5 ml. being added) and heated to about 60° . 2 ml. of freshly-prepared warm 2.5% gelatin solution is added and then, with constant swirling, 3 to 4 ml. of solution of formaldehyde. The mixture is cooled to about 20° , acidified with excess of acetic acid, and 25 ml. of N/10 iodine added. The excess of iodine is titrated with N/10 thiosulphate.

In organic compounds of mercury, the organic matter must first be destroyed by heating with concentrated sulphuric acid, the resulting solution and washings being oxidised with bromine water, with subsequent boiling to remove bromine. Readily volatile or stable alkyl compounds are best decomposed by preliminary treatment with pure bromine in a flask provided with a ground-glass air condenser, the resulting mixture being then heated with sulphuric acid. Prolonged heating with sulphuric acid is avoided by adding a few drops of nitric acid.—M. Fitzgibbon, *Analyst*, 1937, 654.

Results obtained by the iodometric method of Rupp are in general inaccurate. The various modifications suggested, such as the use of ether and alcohol, gelatin, etc., to facilitate solution of the mercury, were found to be only partially effective or inconvenient. High results are probably due to reduction of iodine by formic acid produced from formaldehyde by action of alkali and during the reduction of mercuric salt, and present in formalin. To overcome this defect it was found necessary to filter off the mercury before determining it iodometrically. Filtration is facilitated by precipitating with the finely divided mercury some basic magnesium carbonate, which in no way interferes with the assay process.—H. A. Sloviter *et al.*, *Industr. Engng Chem. (anal. Edn.)*, 1941, 890.

Mayer's Reagent. (*Tanret's Reagent* is identical in composition.) Mercuric chloride 13.546 g., potassium iodide 49.8 g., distilled water to 1 litre. This reagent gives a precipitate with alkaloids.

Formerly, methods of volumetric estimation of alkaloids by means of the above were in vogue, but the composition of the precipitates is variable.

Liquor Hydrargyri Perchloridi (B.P.). Contains 0.095 to 0.105% *w/v* of mercuric chloride. Assayed by reduction with formaldehyde, followed by interaction with excess N/10 iodine and back titration with N/10 sodium thiosulphate.

Toxibellæ Hydrargyri Bichloridi Magnæ (U.S.P. XII). Contain an average of 0.42 g. to 0.52 g. of HgCl_2 , with a sufficient quantity of excipient or diluent. Assayed by the process described for mercuric chloride.

Toxibellæ Hydrargyri Bichloridi Parvæ (U.S.P. XII). Contain an average of 0.11 g. to 0.14 g. of HgCl_2 with a sufficient quantity of excipient or diluent.

Hydrargyri Salicylas (B.P.C. Supp. IV). Contains from 54 to 59.6% of Hg. Residue on gentle ignition at about 300° , not more than 0.2%. Assayed by addition of potassium permanganate to a solution of the substance in N/1 sodium carbonate and after 5 mins. addition of sulphuric acid, followed after a further 5 mins. with ferrous sulphate solution. When the precipitate is dissolved, nitric acid is added and titration made with N/10 ammonium thiocyanate using ferric ammonium sulphate indicator. The U.S.P. XII substance should contain from 54 to 59.5% of Hg; determined by heating with sulphuric and nitric acids on a sand-bath until a colourless mixture results, diluting, and titrating with N/10 ammonium thiocyanate. Hydrargyrum salicylicum, P.G. VI, contains not less than 92% of $\text{C}_6\text{H}_5(\text{OH})\text{COOHg}$.

Hydrargyri Subchloridum (B.P.). $\text{HgCl}=236.1$. Should contain not less than 99.6% of the pure substance. Residue on volatilisation, not more than 0.1%. Assayed by shaking in water with N/10 iodine and potassium iodide and titrating with N/10 sodium thiosulphate. Hydrargyri Chloridum Mite, U.S.P. XII, after drying to constant weight over sulphuric acid, contains not less than 99.6% of HgCl . Determined by interaction with iodine and potassium iodide and titration of the excess iodine with sodium thiosulphate.

Finely Divided Calomel (Duret). Dissolve sodium bicarbonate 6 g. and glucose 10 g. in distilled water 80 ml. Then dissolve separately magnesium chloride cryst. 7.5 g. in water 20 ml. Mix the above and add to a third solution consisting of mercuric chloride 11.5 g., hydrochloric acid 10 drops and water 100 ml. Shake well and allow to stand. Carbon dioxide is evolved. When this slackens, warm on a water-bath until no more gas comes off; wash and dry the precipitate. Yield, 10 g. of a light form of calomel, which may be more active for local use.

Calomel made by this formula is similar to the scaly calomel recommended by Burdon Cooper some years ago for ophthalmic use.

Tabellæ Hydrargyri Chloridi Mitis (N.F. VII). Contain 92.5 to 107.5% of the labelled amount of mild mercurous chloride, including all tolerances. Comply with a test for absence of mercuric chloride. Assayed by washing a weighed quantity of powdered tablets with water, and treating the residue with potassium iodide and excess N/10 iodine, finally titrating with N/10 sodium thiosulphate.

Tabellæ Hydrargyri Chloridi Mitis et Sodii Bicarbonatis (N.F. VII). Contain 92.5 to 107.5% of the labelled amount of mild mercurous chloride for tablets containing more than 15 mg., and 90 to 110% for tablets containing 15 mg. or less of mild mercurous chloride, including all tolerances. Assayed by dissolving in dilute acetic acid and proceeding as for mercurous chloride tablets.

An official quantitative method for the determination of mercurous chloride in tablets is described in *Methods of Analysis (A.O.A.C., 1940, 620)*. A tentative quantitative method for the determination of mercurous chloride in calomel ointment is also described. The soluble fillers in the tablets are dissolved in water acidified with acetic acid, and the insoluble portion collected on an asbestos mat and washed with water, alcohol and ether. In the case of the ointment, the base is extracted with chloroform and the insoluble portion collected on asbestos. In either case the asbestos mat is transferred to a flask and potassium iodide and iodine added; after $1\frac{1}{2}$ hours or when the calomel is dissolved, back titration is made with N/10 sodium thiosulphate, adding an excess and readjusting with N/10 iodine, using starch mucilage as indicator.

Hydrargyri Succinimidum (U.S.P. XII). Dried for 18 hours over sulphuric acid, it contains 49·5 to 51% of Hg, corresponding to not less than 98% of $C_4H_4O_2N_2Hg$; determined by titration with ammonium thiocyanate.

Phenylhydrargyri Nitras (B.P. Add. IV). $C_{12}H_{11}O_4NHg_2 = 634\cdot3$. When assayed by digestion with dilute hydrochloric acid, precipitation as sulphide and drying at 110° , it contains not less than 98% of the pure substance. M.p., 185° to 190° , with decomposition, when heated with a rise of temperature of 5° per minute. A limit of mercuric salts and heavy metals is included. Loss over sulphuric acid, not more than 1%.

Hydrargyrum (B.P.). Hg = 200·6. Assayed by titration of the solution in nitric acid with ammonium thiocyanate; should contain not less than 99·5% of Hg. Residue on volatilisation at about 300° , not more than 0·02%. The U.S.P. XII requires Hydrargyrum to leave not more than 0·01% residue on ignition.

Detection of Mercury. Mercury may be detected by means of diphenylcarbazone, $C_6H_5\cdot NH\cdot NH\cdot CO\cdot N : N\cdot C_6H_5$. The reagent is used as a saturated solution in alcohol. The test solution is rendered neutral or very faintly alkaline (pH 7 to 8) by the addition of sodium acetate (about 1 g. of sodium acetate per 10 ml. of solution is usually sufficient), and 1 drop of reagent per 10 ml. of solution is added. In the presence of mercury a violet or blue coloration is produced. The sensitivity of the reaction is greatly decreased if halides or cyanides be present in considerable quantity. Pb, Cu, Sn, Cd, Ni and Co interfere if only traces of Hg are present. In the case of an organic compound the substance is boiled with hydrochloric acid and potassium chlorate, and pieces of zinc are placed in the solution for 24 hours. The zinc is treated with chlorine water and the test is applied to the liquid obtained.

Detection and Determination with Reinecke's Salt. Mercuric salts in N/10 hydrochloric acid solution gave a voluminous pale red precipitate with Reinecke's salt, $NH_4[Cr(NH_2)_2(SCN)_2]\cdot H_2O$. 2·5γ of the Hg can be detected in 5 ml. after 2 minutes standing. Only gold, silver and thallium compounds interfere. For the determination the solution should contain not more than 0·02 g. of Hg per 100 ml.; moderate amounts of nitric, sulphuric, acetic or tartaric acid do not interfere. The solution is acidified with hydrochloric acid until about N/2, heated nearly to boiling in a steam-bath in a covered beaker and treated, drop by drop, with a filtered, slightly acid solution of the reagent (0·05 g. per 0·01 g. of Hg). After standing a few minutes the precipitate is collected on a sintered glass crucible and well-washed with hot water. The subsequent determination may be carried out volumetrically or gravimetrically. In the volumetric process the precipitate is dissolved in the crucible by adding 0·2 to 0·3 g. of potassium cyanide and hot water, the filtrate and washings being collected in a 400 ml. flask, any chromium hydroxide that separates being dissolved in N/1 hydrochloric acid. Dilute the solution to 100 or 250 ml., add 3 to 7 ml. of strong sulphuric acid and 2 to 3 g. of potassium bromate, and boil for 15 minutes. Oxidation is promoted by the addition of 1 drop of nickel nitrate solution. Bromate is then destroyed by boiling for 20 minutes with 5 to 7 g. of ammonium sulphate and 5 to 6 ml. of N/1 hydrochloric acid; a current of CO_2 helps to remove bromine. Replace from time to time the water lost by evaporation. Cool, add 2 to 3 g. of potassium iodide and titrate with sodium thiosulphate. Each ml. of N/10 thiosulphate is equivalent to 0·0033495 g. of Hg. The gravimetric determination is effected by washing the precipitate with water and alcohol, drying for $1\frac{1}{2}$ hours at 105° to 110° and weighing; it contains 23·96% of Hg. If there is much precipitate it should be collected in a porous porcelain crucible or on filter paper and ignited to chromic oxide. 1 g. of Cr_2O_3 is equivalent to 1·3198 g. of Hg.—C. Mahr, *Z. anal. Chem.*, 1938, 241.

Determination in Organic Compounds. Based on Rupp's iodometric method a new procedure is described. The sample is decomposed by means of persulphate and sulphuric acid, bromide-bromate solution, potassium iodide and sodium hydroxide added and the mercury precipitated by hydrazine sulphate solution. Then sodium bicarbonate and magnesium sulphate are added to

produce magnesium carbonate to facilitate filtration, and the mercury filtered out. The mercury is then estimated by adding bromide-bromate solution, sulphuric acid and potassium iodide and titrating the iodine with sodium thio-sulphate. The method is applicable in the presence of halogens.—H. A. Sloviter *et al.*, *Industr. Engng Chem. (anal. Edn.)*, 1941, 890.

Hydrargyrum cum Creta (*B.P. Add. I*). Contains from 31 to 35% of mercury. Assayed by boiling with nitric acid and water, cooling, diluting, adding potassium permanganate solution to produce a permanent pink colour, decolorising with ferrous sulphate, and titrating with standard ammonium thiocyanate. Hydrargyrum cum Creta, *U.S.P. XII*, contains from 36 to 40% of Hg.

Mercury in a finely divided state is rapidly oxidised and a limit for mercuric mercury should have been included.—*Pharm. J.*, ii/1932, 106.

Pilula Hydrargyri (*B.P.*). Contains 32 to 34% of mercury.

Unguentum Hydrargyri (*B.P. Add. VI*). Assayed by boiling with nitric acid and water, filtering, adding potassium permanganate to a faint pink coloration, followed by decolorisation with ferrous sulphate and titration with N/10 ammonium thiocyanate; it should contain from 29 to 31% of mercury. The base now consists of a mixture of oleated mercury, wool fat, white beeswax and white soft paraffin. Unguentum Hydrargyri Dilutum, *B.P. Add. IV*, assayed similarly, contains 9.5 to 10.5% of Hg. Unguentum Hydrargyri Forte, *U.S.P. XII*, contains 47.5 to 52.5% of Hg, and Unguentum Hydrargyri Mite, *U.S.P. XII*, contains 9 to 11% of Hg; the Pommade Mercurielle, *Fr. Cx.* 1937, after washing with acidified alcohol-ether mixture leaves 50% of mercury. The dilute ointment contains 12.5%.

Unguentum Hydrargyri Nitratis Forte (*B.P.*). Contains not less than the equivalent of 6.7% of Hg. Assayed by digestion with sulphuric and nitric acids, precipitation as sulphide, finally drying at 110° and weighing.

Assay of Mercurial Ointments. The method recommended for the assay of mercury or one of its compounds in ointments, when it is distributed through, but not chemically combined with, the basis, consists of dissolving the basis in xylol in a centrifuge tube, and centrifugally separating out the suspended solid. Most of the liquid is now syphoned off, and the process repeated twice with fresh portions of solvent, replacing the xylol after the first time with light petroleum. A layer of alcohol is now interposed between the deposited solid and the petroleum layer. The mixture is centrifuged again, and the petroleum layer, along with some of the alcohol, drawn off. Either the residual alcohol is now evaporated off, the solid dissolved in nitric acid, transferred to a flask and titrated with ammonium thiocyanate, or the mixture is shaken, the alcoholic suspension rinsed into a flask, and the assay of the salt completed by the official or other suitable process.—W. R. Heading, *Quart. J. Pharm.*, 1934, 406.

The second report of the Commission Internationale des Spécialités includes an agreed method for the determination of mercury in ointments and plasters in which after separation from the base by means of a solvent the mercury compound is oxidised to the mercuric state with sulphuric and nitric acids and determined by precipitation as sulphide or titration with thiocyanate.—*Bull. Féd. int. Pharm.*, 1938, 56.

Method for Strong Ointment of Mercuric Nitrate. Heat the ointment with 50% aqueous potassium hydroxide for about 35 minutes in the presence of zinc dust. The mercury is set free first as mercuric oxide, which is then

reduced to metal by the hydrogen generated from the action of the alkali on the zinc. An amalgam is formed with the excess of zinc, and the mercury is thus obtained quantitatively in a granular form, which may easily be freed from soap by decantation, filtration and washing. The amalgam is next dissolved in nitric acid, and the mercury determined by means of ammonium thiocyanate, the presence of zinc as nitrate being quite without effect.—W. R. Heading, *Quart. J. Pharm.*, 1934, 413.

The official method may be modified with advantage by substituting titration with standard thiocyanate solution for gravimetric determination of the mercury as sulphide.

To about 5 g. in a long-necked flask of about 250 ml. capacity add 35 ml. of sulphuric acid, and heat cautiously until the mixture darkens. Add gradually 5 ml. of fuming nitric acid, rotating the flask to assist the escape of evolved gases. Heat, and maintain just below the boiling point. Repeat several times the addition of fuming nitric acid and the heating, until an almost colourless solution remains. Cool, add a slight excess of solution of potassium permanganate and heat to boiling. Add sufficient solution of hydrogen peroxide to make the solution colourless, cool and dilute to 250 ml. Titrate 100 ml. with N/10 ammonium thiocyanate, using solution of ferric ammonium sulphate as indicator.—C. H. Hampshire and G. R. Page, *Quart. J. Pharm.*, 1935, 75.

Methods involving digestion with sulphuric and nitric acids and saponification by aqueous potash give low results and the following method in which saponification is carried out with an aqueous-alcoholic solution is recommended.

To 2 g. of ointment in a 250 ml. conical flask add 10 g. potassium hydroxide pellets, 2 g. zinc dust, 10 ml. water and 10 to 20 ml. industrial methylated spirit, and boil for fifteen minutes over a small flame under a reflux condenser of the Liebig type, cautiously adding through the condenser a mixture of 3 ml. of formaldehyde solution and 50 ml. of water and then heat just to boiling and remove the flame. Filter off the hot soap solution through a paper pulp filter in a Gooch crucible, wash the amalgam with water by decantation, transfer the paper pulp to the flask and refit to the condenser. Pour through the condenser 20 ml. of water and then gradually 20 ml. of nitric acid, transferring any amalgam adhering to the crucible with a few drops of nitric acid. Heat the solution to remove the greater part of the nitrous fumes, cool thoroughly, oxidise with permanganate, decolorise with a drop of hydrogen peroxide and titrate with N/10 ammonium thiocyanate using iron alum as indicator.—*Quart. J. Pharm.*, 1933, 431.

The method of reducing the mercury compound to metal and amalgamating with zinc dust or filings is modified for oleated mercury, red mercuric iodide ointment, dilute ointment of mercuric nitrate and ointment of oleated mercury. The value of ethyleneglycol monoethylether as a menstruum for saponification in the assay of mercurial ointments containing paraffin is demonstrated.—G. J. W. Ferrey, *Quart. J. Pharm.*, 1939, 413.

A tentative method for the assay of mercury in ointment of mercuric nitrate is described in *Methods of Analysis* (A.O.A.C., 1940, 622).

Unguentum Mercuriale (B.P.C.). Contains 33·3% of Unguentum Hydrargyri diluted with lard.

Hydrargyrum Ammoniatum (B.P.). NH_2HgCl = 252·1. Contains not less than 97% and not more than the equivalent of 100·5% of NH_2HgCl . Residue on ignition at a low red heat, not more than 0·2%. Assayed by treatment with potassium iodide and titration to methyl orange with N/10 hydrochloric acid. The U.S.P. XII substance is assayed for Hg content by precipitation as sulphide, and should contain HgNH_2Cl equivalent to from 78 to 80% of Hg.

Hydrargyrum præcipitatum album, P.G. VI, contains the equivalent of not less than 98·3% of NH_2HgCl . Hydrargyrum præcipitatum album, P. Helv. V, is assayed by the following process:—Mix 0·2 g. with about 50 ml. of water and 2 g. of sodium thiosulphate and shake frequently during 10 minutes until dissolved; add 2 or 3 drops of methyl orange and titrate with N/10

hydrochloric acid. 1 ml. of N/10 HCl is equivalent to 0.012604 g. of HgClNH_2 .

Assay of Ung. Hydrarg. Ammon. B.P. 1932. Weigh 3 g. of sample in a 250 ml. conical flask, add 25 ml. of xylol and gently warm to dissolve the base. Add 10 ml. of glacial acetic acid and 2 to 3 g. of potassium iodide dissolved in 10 ml. of water, using this solution to wash down the walls of the flask. Shake with a swirling motion for a few seconds until the aqueous solution is clear and pale brown in colour. Add 2 g. of zinc filings, reflux gently for fifteen minutes, wash down the condenser with 20 ml. of warm water and boil for a further five minutes. Remove the flame, allow to cool somewhat and add 150 ml. of water. Decant the xylol and aqueous solutions through a filter under slight suction. Wash the amalgam by decantation with xylol and water until the washings give no reaction for iodide. Dissolve the amalgam in dilute nitric acid, boil gently to remove nitrous fumes, cool, oxidise with permanganate, decolorise with a drop of solution of hydrogen peroxide and titrate with N/10 or N/20 ammonium thiocyanate. The method is also applicable to ointment of mercurous chloride, using 1 to 1.5 g. of sample, and to ointment of mercuric oxide, using 2 g. of a 10% ointment or 5 g. of a 1% ointment.—G. J. W. Ferrey, *Quart. J. Pharm.*, 1941, 168.

Hydrargyrum Oleatum (B.P.). Should contain the equivalent of from 19 to 21% of yellow mercuric oxide. Assayed gravimetrically by precipitation as sulphide from a solution in benzene, glacial acetic acid and alcohol. Oleatum Hydrargyri, U.S.P. XII, contains the equivalent of 24 to 26% of HgO ; determined by the method for Massa Hydrargyri, by digestion in a Kjeldahl flask with sulphuric and nitric acids; after dilution potassium permanganate is added to the warm solution to produce a slight pink colour, this being discharged, after cooling, with oxalic acid, nitric acid added and titrating with N/10 ammonium thiocyanate. The preparation also contains a small amount of uncombined oleic acid.

Mercurochromum (B.P.C.). Loses on drying *in vacuo* at 50° over sulphuric acid, not more than 10% and then contains from 25 to 28% of Hg and from 21 to 23% of Br. Limit tests for free mercury, soluble mercury salts and sodium are described. Hg is estimated by separation as the zinc amalgam, which is then dissolved in nitric acid and treated with urea, the oxidised solution being titrated with N/10 ammonium thiocyanate; assayed for Br content by titration of the product of the fusion with potassium nitrate and potassium and sodium carbonates, with silver nitrate and ammonium thiocyanate. The B.P.C. '34 introduced a standard consisting of a quantity of mercurochrome kept by the Pharmaceutical Society and supplied on request. It is required that mercurochrome for intravenous injection shall be not more toxic than this standard sample. Manufacturers preparing mercurochrome are expected to determine the average lethal dose of the standard mercurochrome for mice in the conditions of their own laboratory. This dose, which kills 50% of mice, must be determined with care. Mice are then injected with the unknown sample, using the above determined dose. Ten mice are first injected, and if not more than two die, the sample passes the test. If more than two die, ten more are injected. If out of the twenty injected not more than eight die, the sample passes the test. If more than eight but less than fifteen die, ten more mice are

injected, and the sample passes the test if the number of deaths is not greater than fifteen out of the thirty mice injected. Merbrominum, *N.F. VII*, when dried at 110° , contains from 24 to 26.7% of Hg and from 18 to 21.3% Br. It is assayed gravimetrically for Br by fusion with anhydrous sodium carbonate, magnesium oxide and reduced iron followed by extraction with water and precipitation of silver bromide. Hg is determined by oxidation with potassium permanganate and sulphuric acid, decolorisation with oxalic acid and precipitation of mercuric sulphide.

Alkaline-Permanganate Oxidation Assay. The results obtained by the B.P.C. method for the assay of mercury show an appreciable experimental variation. The following alkaline-permanganate oxidation method appears to give more reliable and consistent results:—

Dissolve 0.5 g. of mercurochrome in 50 ml. of water in a 600 ml. beaker, add 10 ml. of 40% caustic soda solution and 2 to 3 g. of potassium permanganate, and boil gently over a micro-burner for 1 minutes. Cool slightly and add gradually a cooled mixture of 10 ml. of concentrated sulphuric acid and 200 ml. of water, avoiding loss by spurling by covering the beaker with a clock glass. Allow to cool, and add in small portions, with rapid stirring, a slight excess of 10 vols. hydrogen peroxide. Replace over the micro-burner, add excess of 5% potassium permanganate solution and ensure an excess after boiling gently for 10 minutes. Add dilute oxalic acid solution, drop by drop, until a water-white solution is obtained. Precipitation of the mercury as sulphide with subsequent filtration and weighing is carried out by the usual routine.—R. F. Corran and F. E. Rymill, *Quart. J. Pharm.*, 1935, 340.

Acid-Permanganate Oxidation, Assay.—*Methods of Analysis (A.O.A.C., 1940, 621).*

The mercury content may be rapidly determined by the following method. Half to 1 g. of mercurochrome is weighed out into a 250 ml. conical flask and dissolved in 20 ml. of water. 5 g. of caustic potash pellets and 2 g. of zinc filings are added and the mixture boiled under reflux condenser for at least 15 minutes. The flame is then removed and the condenser washed down with 50 ml. of water. The amalgam is filtered off by decantation, washed with water and dissolved in a mixture of 20 ml. of nitric acid and 20 ml. of water. After gently boiling off the nitrous fumes, the solution is cooled, treated with potassium permanganate, decolorised with a drop of hydrogen peroxide and titrated with N/10 ammonium thiocyanate. The results so obtained are in agreement with those given by the Codex method, and it is probable that both methods account for the whole of the mercury in the samples of mercurochrome assayed.—G. J. W. Ferrey, *Quart. J. Pharm.*, 1940, 210.

Mersalylum (B.P. Add. I). $C_{13}H_{16}O_6NHgNa = 505.7$. Assayed for nitrogen by the Kjeldahl method, it contains 2.5 to 2.8% of N, and for mercury by digestion for 3 hours with water and hydrochloric acid, precipitation with hydrogen sulphide, washing the mercury sulphide on a Gooch crucible with hydrogen sulphide solution, alcohol and carbon disulphide and drying at 110° , it contains 38.5 to 40.5% of Hg, both calculated on the substance dried in a vacuum desiccator. Limit tests for mercuric salts and heavy metals, chloride, sulphate, and foreign organic matter are included. Mersalylum, *U.S.P. XII*, complies with the same standard for Hg. Loss on drying over sulphuric acid, not more than 7%.

HYDRASTIS

Hydrastis (B.P.C.). Foreign organic matter, not more than 2%. Acid-insoluble ash, not more than 3%. Hydrastis, *N.F. VII*,

should contain not more than 4% of foreign organic matter; it should yield not less than 2.5% of ether-soluble alkaloids of Hydrastis and not more than 3% of acid-insoluble ash. It is assayed gravimetrically by maceration with ether, shaking an aliquot part with acid and transferring the alkaloids to ether, finally drying at 100°. Rhizoma Hydrastis, *P.G. VI*, yields not less than 2.5% of hydrastine; assayed by maceration with ether and ammonia, addition of petroleum ether and water, filtration and evaporation of an aliquot part of the filtrate, finally dissolving in excess N/10 hydrochloric acid and titrating with N/10 potassium hydroxide to methyl orange indicator. Rhizoma Hydrastis, *P. Helv. V*, should also contain 2.5% of hydrastine by a process similar to the *N.F. VII* assay, using dilute hydrochloric acid for the extraction and ether and petroleum ether for the final extraction, and drying the hydrastine for 1 hour at 103° to 105°.

Since 1927 the highest yield of total alkaloids has steadily declined. In 1927 the highest yield of total alkaloids was 3.12% and the lowest 3.02% (by the *U.S.P. X* method). In 1936 the corresponding figures were 2.59 and 1.74% respectively. The *B.P.C.* (for the extract), *U.S.P. X* and *N.F. VI* methods of assay give different results. Two samples, giving respectively 2.73 and 1.74% by the *U.S.P. X* method, gave 2.56 and 1.34% by the *B.P.C.* method.—W. A. N. Markwell, *Chem. & Drugg.*, i/1937, 90.

Extractum Hydrastis Liquidum (*B.P.C.*). Assayed by addition of diluted potassium iodide solution and extraction with ether of an aliquot part of the filtrate rendered ammoniacal, finally drying the residual alkaloid on a water-bath; should yield 1.9 to 2.1% *w/v* of hydrastine. Fluidextractum Hydrastis, *N.F. VII*, contains 2.25 to 2.75% *w/v* of ether-soluble alkaloids of hydrastis; determined by extraction of total alkaloid with ether from ammoniacal mixture, transferring to acid and back to ether, and drying the final alkaloids at 100°.

Extractum Hydrastis (*B.P.C.*). Contains 7.5 to 8.5% of hydrastine. Extractum Hydrastis, *N.F. VII*, contains 9 to 11% of ether-soluble alkaloids.

Hydrastinae Hydrochloridum (*B.P.C.*). $C_{21}H_{21}O_6N, HCl = 419.6$. Loss at 100°, not more than 6%. Ash, not more than 0.1%. Limit tests for hydrastinine and berberine are included. The *N.F. VII* also includes these tests, and limits the moisture to 2% on drying over sulphuric acid.

Hydrastinine Hydrochloridum (*B.P.C.*). $C_{11}H_{12}O_2NCl = 223.5$. Ash, not more than 0.1%. Tests for limit of foreign alkaloids and hydrastine are described.

HYDROGENII PEROXIDUM

Liquor Hydrogenii Peroxidi (*B.P.*). Contains 2.5 to 3.5% *w/v* of H_2O_2 , corresponding to about ten times its volume of available oxygen. Residue on evaporation on a water-bath, not more than 0.2% *w/v*. Complies with limit tests for barium and acidity. Assayed by titration with potassium permanganate.

Liquor Hydrogenii Dioxidii, *U.S.P. XII*, contains 2·5 to 3·5% *w/v* of H_2O_2 .

P.G. VI gives method of estimation by titrating iodine liberated from potassium iodide.

HYOSCYAMUS

Hyoscyamus (B.P.). Contains not less than 0·05% of the alkaloids of hyoscyamus, calculated as hyoscyamine, not more than 2% of foreign organic matter and not more than 1% of stems more than 5 mm. in diameter. Ash limit, 20%. Acid-insoluble ash limit, 12%. Assayed by the process of the *B.P. Add. I*.—Hyoscyamus is macerated and percolated with ether-alcohol (4 : 1) with the addition of ammonia, the percolate extracted with N/2 hydrochloric acid and alcohol (4 : 1); the mixed acid liquids are cleaned with chloroform, made ammoniacal and the alkaloid, extracted as quickly as possible with chloroform, finally dried at 80° for 2 hours to remove volatile bases and titrated to methyl red or cochineal. Hyoscyamus, *U.S.P. XII*, is assayed either by maceration with ammonia solution, alcohol and ether, and continuous extraction with ether, or by maceration with ammonia solution, ether and chloroform, and percolation with ether-chloroform; either extractive is evaporated to low bulk, adding N/10 sulphuric acid and water towards the end of the evaporation and the acid liquids made ammoniacal and extracted with chloroform; the final alkaloidal residue is heated for 15 minutes on a water-bath, dissolved in chloroform, again heated and this operation repeated a third time; not less than 0·04% of alkaloids should be present. It contains not more than 25% of its stems, none being more than 7 mm. in diameter; acid-insoluble ash, not more than 12%.

Folia Hyoscyami, P.G. VI, yields not less than 0·07% of hyoscyamine. *Folium Hyoscyami, P. Helv. V*, consists of the leaves of the flowering plant of *Hyoscyamus niger* Linn., without stem, fruits or flowers, and contains not less than 0·05% of alkaloid. It may yield up to 20% of total ash and up to 6% of acid-insoluble ash. *Herba Hyoscyami mutici, P. Helv. V*, from *Hyoscyamus muticus* Linn., contains not less than 0·8% of alkaloids and is used for making extract and tincture of hyoscyamus.

The root is richest in total alkaloids and the annual plant is, if anything a trifle richer than the biennial leaves either of the first or second year: thus, biennial root, 0·16%; leaves, biennial first year, 0·059 to 0·069%; leaves and tops, second year, 0·065 to 0·068%; leaves and tops, annual, 0·064 to 0·07%.—S. Jensen, *Pharm. J.*, i/1915, 98.

Extractum Hyoscyami Liquidum (B.P.). Contains 0·045 to 0·055% *w/v* of alkaloids of Hyoscyamus.

Extractum Hyoscyami Siccum (B.P.). Contains 0·27 to 0·33% of alkaloids of Hyoscyamus. Extractum Hyoscyami, *U.S.P. XII* yields 0·135 to 0·175% of alkaloids.

Tinctura Hyoscyami (B.P.). Contains 0·0045 to 0·0055% *w/v* of alkaloids of Hyoscyamus, calculated as hyoscyamine. Tinctura

Hyoscyami, *U.S.P. XII*, contains 0.0034 to 0.0046% *w/v* of alkaloids.

Hyoscinae Hydrobromidum (B.P.). $C_{17}H_{21}O_4N \cdot HBr \cdot 3H_2O = 438.1$. M.p., after drying at 100° , 194° to 196° . Specific rotation determined in 5% *w/v* aqueous solution of the anhydrous salt, -24° to -26° . Loss at 100° , 12 to 13%. Ash, not more than 0.1%. Scopolaminae Hydrobromidum, *U.S.P. XII*, loses not more than 13% at 100° ; tests for apoeptropine and for morphine are included.

Tabellae Scopolaminae Hydrobromidi (N.F. VII). Tablets of 1.2 mg. or more contain 91 to 109%, and tablets of less than 1.2 mg. contain 88 to 112%, of the labelled amount of scopolamine hydrobromide, including all tolerances. Assayed by extracting with chloroform from an ammoniacal solution, adding 10 ml. of alcohol for each 50 ml. of chloroform to the combined chloroformic extracts, evaporating and dissolving the residue in alcohol and N/20 sulphuric acid, finally back titrating with N/50 sodium hydroxide using methyl red indicator.

Hyoscyaminae Hydrobromidum (B.P.C.). $C_{17}H_{23}O_3N \cdot HBr = 370.1$. Loss at 100° , not more than 1%. Ash, not more than 0.1%.

Hyoscyaminae Sulphas (B.P.C.). $(C_{17}H_{23}O_3N)_2 \cdot H_2SO_4 = 676.5$. M.p., not below 203° . Loss at 100° , not more than 1%. Ash, not more than 0.1%.

ICHTHAMMOL

Ichthammol (B.P.). The dried substance contains not less than 10.5% *w/v* of organically combined sulphur, and not more sulphur in the form of sulphates than one-fourth of the total sulphur. Loss at 100° , not more than 50% by weight. Residue on ignition and re-ignition with sulphuric acid, not more than 0.3%. Assayed for total sulphur by gentle ignition with sodium carbonate and copper nitrate, dispersion of the ichthammol in the sodium carbonate having been effected by evaporation with chloroform, followed by precipitation as barium compound and ignition: assayed for sulphates by precipitation of a solution with cupric chloride solution, filtration and precipitation with barium chloride; organically combined sulphur is obtained by the difference between these two percentages. Ichthammol, *N.F. VII*, by distillation with sodium hydroxide, yields not less than 2.5% of ammonia; by precipitation of the sulphate as barium salt from an alcohol and alcohol-ether extractive it yields not more than 8% of ammonium sulphate; by oxidation with potassium chlorate and nitric acid and final precipitation as barium sulphate, not less than 10% of total sulphur should be indicated.

Ichthyolsulfonate d'Ammonium, *Fr. Cx. 1937*, contains 8 to 12% of total sulphur, determined by ignition with sodium carbonate and potassium nitrate and precipitation as barium sulphate; sulphur as ammonium sulphate should be 1.25 to 1.50%, determined by treatment of an aqueous solution with egg white and hydrochloric acid and precipitation of the cold filtrate with barium salt. Ammonium sulfobituminosum, *P. Helv. V*, contains

1.2 to 4.2% of total ammonia, not more than 1.7% of sulphur as sulphate, and not less than 7.25% of total sulphur. The difference between the percentage of total sulphur and the sum of the sulphur as sulphate and the sulphur as sulphonie sulphur, gives the percentage of organically combined sulphur, which should not be less than 4%. Total ammonia is determined by distillation with magnesia into excess standard acid; ammonium sulphate is determined by precipitation of a solution of 3 g. of the substance in about 250 ml. of water with 20 ml. of a white of egg solution and 3 portions of 10 ml. of dilute hydrochloric acid, adjusting to 500 ml., filtration and precipitation of an aliquot part with barium chloride; total sulphur is determined by digestion with nitric acid and potassium chlorate during 3 to 4 hours and until reduced to low bulk, evaporation with hydrochloric acid and precipitation of barium sulphate.

INSULINUM

Insulinum (*B.P. Add. I and VI*). Complies with tests for sterility, and the solution contains 20 units per millilitre. Controlled by regulations of the Therapeutic Substances Act, 1925. The unit is the specific activity contained in such an amount of the standard preparation as the Medical Research Council may indicate as the quantity exactly equivalent to the unit accepted for international use. Crystalline insulin contains approximately 24,000 units per gramme. The label on the container or packet must state the date of manufacture and the date after which the preparation should not be used. The solution should be stored at as low a temperature as possible above the freezing-point and not exposed to a temperature above 20°. Insuline, *Fr. Cx.* 1937, should not contain more than 12 g. of nitrogen for 100 g. insulin containing 15 units per mg. Complies with tests for amino-acids and inactive proteins. *Injectio Insulini, U.S.P. XII*, is standardised to contain in 1 ml. 20, 40, 80 or 100 U.S.P. Insulin Units. The label on the container states the number of units per ml. and the expiration date which is not more than 2 years after the date of supply by the manufacturer.

Biological Methods of Assay

Rabbit Method. The rabbit method described in the *B.P.* depends on the observation that a dose of about 1 unit of insulin injected under the skin of a fasting rabbit causes a fall in the blood sugar which is greatest after 2 to 3 hours, the concentration returning to the normal value after 5 hours. If the initial percentage of blood sugar is observed, and also the percentage at the end of each hour for 5 hours after injection, a figure for the hypoglycæmic effect can be calculated. This is done by taking the average figure for the 5 hours after injection, and subtracting it from the initial figure. The result is then expressed as a percentage

of the initial figure and is called the percentage blood sugar reduction.

In performing the test, twelve rabbits of about 2 kg. weight, which have been without food for 24 hours, are used. Six are injected with 1 unit of the standard preparation, and six with a quantity of the unknown sample expected to contain 1 unit. The percentage blood sugar reduction is then determined for each rabbit. Two days later, the experiment is repeated on the same animals; the rabbits which before received the standard now receive the unknown, and those which received the unknown now receive the standard. There are now two figures available for each rabbit, namely the percentage blood sugar reductions when injected with the standard and those when injected with the unknown. The average figure given by all the rabbits for the percentage blood sugar reduction after injection with the standard is then compared with that after injection with the unknown preparation. If the dose of the unknown preparation was correctly chosen the two figures should be equal. If they differ appreciably the experiment must be repeated, using a different dose of the unknown.

Mouse Method. The mouse method depends on the appearance of convulsions in mice after they are injected with insulin. A large group of mice is taken, of which one half receives a dose of the unknown preparation, the other half receiving the standard. The percentage of mice in each group in which convulsions occur is then determined, and, from the result, the potency of the unknown can be stated in terms of that of the standard.

The mice are prepared by being kept without food overnight. They are injected and placed immediately in a thermostat kept at a temperature which is usually 29° or 34° . Symptoms, which may be those of collapse or of convulsions, occur within 2 hours, and the proportion of mice showing symptoms is determined. In deciding the potency of the unknown preparation in relation to that of the standard, two doses of the standard are used, one of which causes a higher percentage of convulsions than that caused by the unknown, while the other causes a lower percentage. The dose of the unknown can then be equated to the mean of the two doses of the standard. Alternatively, the relative potency of unknown and standard can be determined from the percentages of convulsions by using a previously determined curve relating dose to percentage convulsions.

A modification of the mouse method for the assay of insulin is described, enabling several unknown preparations to be assayed in a single test. Corrections for use in the simple method of calculation are described, so as to conform more closely with statistical theory. The question of weighting is discussed. —H. P. Marks, *Quart. J. Pharm.*, 1940, 344.

IODUM

Iodum (B.P.). $I=126.9$. By titration with N/10 sodium thiosulphate, it contains not less than 99.5% of I. Residue on volatilisation on a water-bath, not more than 0.05%. A standard of 99.8% of I is required by the *U.S.P. XII*.

Manufacture. The sea-weeds vary enormously in iodine content, and only *L. digitata* ("Tangle Stem") and *L. stenophylla* are worth burning for kelp. The varieties known collectively as *drift kelp* are richer than cut kelp. If properly burnt, the drift weed ought to yield 25 to 30 lb. of iodine per ton. 12 lb. per ton is, however, above the average for ordinary drift kelp. Very good Scotch kelp yields potassium sulphate 14%, potassium chloride 18%, sodium chloride 14%, sodium carbonate 4%, and iodine $14\frac{1}{2}$ lb. per ton. When burnt into "loose ash" these figures are respectively 13%, 18%, 6.8%, 3.4% and 28 lb.

The mineral known as **caliche**, the crude sodium nitrate of Peru and Chili, forms now by far the most important source of iodine. It is present in the form of iodate. Sodium bisulphite is used to precipitate the iodine from the mother liquors from which the sodium nitrate has been crystallised.

Oleum Iodisatum (*B.P. Add. I*). An iodine addition product of poppy-seed oil (stored in previously sterilised containers sealed to exclude bacteria), containing 39 to 41% of combined iodine. Determined by refluxing with glacial acetic acid and zinc powder for 1 hour, diluting and filtering; the cooled filtrate after addition of hydrochloric acid and potassium cyanide solution is titrated with M/20 potassium iodate, using starch mucilage as indicator. A limit of chloro-iodine compounds is included—sodium iodide is added to a solution in acetone and allowed to stand in the dark for 30 minutes with occasional shaking, the mixture then diluted and titrated with N/10 sodium thiosulphate. Free iodine in a solution in chloroform shaken with a potassium iodide solution is titrated with standard sodium thiosulphate. Complies with limit tests for acidity and mercury and with tests for sterility. **Oleum Iodatum**, *U.S.P. XII*, contains 38 to 42% of organically combined iodine, as shown by titration with M/20 potassium iodate of the filtrate obtained by igniting with sodium carbonate, extracting with water, acidifying and adding an equal volume of hydrochloric acid; forms a clear solution with 10 volumes of petroleum ether; ash, not more than 0.1%.

Chlorinated Iodised Oil. By the following process one molecule of iodine and one molecule of chlorine in an adjacent position are introduced into the unsaturated linkage of the fatty acids of the oil. The chlorine stabilises the iodine combination. 1000 g. of oil (vegetable or animal) is mixed with 2000 ml. of denatured alcohol, a few drops of phenolphthalein solution and sufficient N/2 sodium hydroxide to give a pink colour. The alcohol is siphoned off, 2000 ml. of water added and the mixture well stirred. The water is removed and the oil washed with a further 2000 ml. of water. A solution of iodine monochloride is prepared by adding with vigorous stirring in one rapid stream 720 ml. of hydrochloric acid to a solution of 357 g. of potassium iodide and 239 g. of potassium iodate in 720 ml. of water. Stirring is continued until the iodine monochloride first precipitated redissolves. A solution of the purified oil in ether (or other solvent) is gradually added to the iodine monochloride solution with constant stirring, the temperature being maintained at 20°–25°. The iodine monochloride is faintly yellow or almost white after completion of the reaction. The mixture is allowed to stand, the upper aqueous layer removed, 500 ml. of ethylene dichloride added, and the mixture is washed with running tap water until all hydrochloric acid is removed. The ethylene dichloride solution is separated from the water as completely as possible, treated with 750 ml. of dehydrated alcohol and rendered just alkaline to phenolphthalein by adding 4% alcoholic sodium hydroxide. The mixture is allowed to stand, the alcohol removed, the oil well washed, dried over anhydrous sodium sulphate, treated with activated charcoal and filtered, and the solvent removed by evaporation *in vacuo* at first without heat to remove ether, then at 55° for 6–8 hours to remove ethylene dichloride. Stability can be increased by adding $\frac{1}{2}$ –1% of purified non-iodised oil to absorb any free iodine liberated.—F. R. Greenbaum, *Amer. J. Pharm.*, 1937, 407.

Liquor Iodi Aquosus (*B.P.C.*). Prepared to contain 5% *w/v* of iodine and 7.5% *w/v* of potassium iodide. **Liquor Iodi Aquosus**, *B.P. Add. I*, contains 4.9 to 5.1% of iodine and 9.8 to 10.2% of potassium iodide. **Liquor Iodi Fortis**, *U.S.P. XII*, contains 4.5 to 5.5% *w/v* of I, and 9.5 to 10.5% *w/v* of KI. Assayed for iodine by titration with N/10 potassium arsenite. Hydrochloric

acid is then added to the titration mixture and potassium iodide determined by titration with M/20 potassium iodate, correction being made for the iodine present by calculation from the first titration.

Determination of Free and Combined Iodine in Solution. The following method avoids the necessity for shaking in a stoppered bottle and has the advantage that both free and combined iodine are determined on the sample. The iodide is titrated with iodate in the presence of potassium cyanide and hydrochloric acid using starch, the iodine cyanide formed is subsequently decomposed by adding potassium iodide, and the liberated iodine is titrated with N/10 sodium thiosulphate. If a ml. of potassium iodate are required, and b ml. of N/10 thiosulphate, the weight of combined iodine is equal to $0.0031725 (5a-2b)$ g. and that of free iodine is equal to $0.006345 (2b-3a)$ g. The process is applicable to each of the four pharmacopœial iodine solutions. For aqueous solution of iodine the formula should

be modified by multiplying by the factor $\frac{166}{126.9}$.—C. Morton and F. R. C.

Bateson, *Quart. J. Pharm.*, 1937, 498.

Liquor Iodi Decoloratus. No evidence of the presence of iodate could be found. Iodoform was found in fresh samples but not in older ones. It was not possible to demonstrate the presence of a formate.—C. Morton and F. R. C. Bateson, *Quart. J. Pharm.*, 1937, 500.

Liquor Iodi Fortis (B.P.). Contains from 9.8 to 10.2% w/v of iodine and 5.8 to 6.2% of potassium iodide. Iodine content estimated by titration with N/10 sodium thiosulphate, and potassium iodate in presence of 40% of hydrochloric acid, and subtracting one half the thiosulphate titration. Alcohol content, 76 to 79% v/v of ethyl alcohol, decolorising with sodium thiosulphate and adding sodium hydroxide before distillation. *Tinctura Iodi, U.S.P. XII*, contains 6.8 to 7.5% w/v of I and 4.7 to 5.5% w/v of KI; alcohol content 83 to 88% v/v . Assayed for iodine by titration with potassium arsenite and for potassium iodide by titration with M/20 potassium iodate. *Tinctura Iodi Fortior, N.F. VII*, contains 16 to 17% w/v of I and 3 to 4% w/v of KI.

Liquor Iodi Mitis (B.P.). Iodine content, 2.45 to 2.55% w/v ; potassium iodide content, 1.45 to 1.55% w/v . Alcohol content, 85 to 88% v/v of ethyl alcohol. *Tinctura Iodi Mitis, U.S.P. XII*, contains 1.8 to 2.2% w/v of I and 2.1 to 2.6% w/v of NaI. *Liquor Iodi, U.S.P. XII*, is an aqueous solution of the same strengths.

Liquor Iodi Simplex (B.P.). Simple solution of iodine is prepared with 9% w/v of iodine in alcohol (95%). Owing to rapid reduction of the iodine it is standardised to contain from 8.8 to 9.2% w/v of total iodine.

When simple solution of iodine is stored the content of free iodine becomes constant in 8 months. The acidity of the solution still increases slightly after the free iodine content has become constant. A solution assaying 8.78% w/v of free iodine 7 days after preparation reached equilibrium at 7.17% of free iodine after storage for 8 months.—G. R. Page, *Quart. J. Pharm.*, 1935, 81.

Unguentum Iodi Denigrescens (B.P.C.). Prepared with arachis oil and yellow soft paraffin to contain 5% w/w of iodine. *Unguentum Iodi, B.P.C.* '34, contains 4% of iodine with potassium iodide and water in simple ointment.

Unguentum Iodi, U.S.P. XII, is assayed for total iodine content (6.5 to 7.5%) by ignition with potassium carbonate, extraction with water, oxidation with permanganate and just decolorising with alcohol, addition of potassium iodide and sulphuric acid to an aliquot part of the filtered liquid, and titration with sodium thiosulphate.

Assay. The following method for the determination of iodine in iodised oils may be used for the assay of iodine in non-staining iodine ointments:—About 1 g. of the sample, accurately weighed, is boiled for one hour under a reflux condenser with 10 ml. of glacial acetic acid and 1 g. of zinc filings. 30 ml. of hot water is added down the condenser tube, the liquid is filtered through a plug of wet cotton wool, and the flask and filter are washed with two portions of 20 ml. of water. The combined filtrate and washings (the filtrate need not be clear) are cooled, 100 ml. of hydrochloric acid is added, and the liquid titrated with M/20 potassium iodate, chloroform being added as indicator towards the end of titration, and titration being continued until, after vigorous shaking, the chloroform is colourless, the aqueous liquid being clear yellow. Each millilitre of M/20 potassium iodate is equivalent to 0.01269 g. of I.—T. T. Cocking and G. Middleton, *Quart. J. Pharm.*, 1931, 176.

The free and combined iodine in stainless or ordinary iodine ointments may be determined by distilling 2 g. of the sample with a little powdered pumice and 100 ml. of strong sulphuric acid in a 400 ml. distilling flask connected to an air condenser and a bulb-tube dipping under 50 ml. of water. Boil gently for 3 to 4 hours, rinse the condenser and tube with water, filter the distillate and washings to remove organic matter which distils over, acidify with nitric acid and precipitate with silver nitrate. Boil, collect, wash, dry and weigh.—F. W. Edwards, E. B. Parkes and H. R. Nanji, *Analyst*, 1935, 748.

A tentative method for the determination of free iodine in iodine ointment is described in *Methods of Analysis (A.O.A.C., 1940, 619)*.

DETERMINATION OF IODINE IN BIOLOGICAL SUBSTANCES

Report of the Medical Research Council: C. O. Harvey, *Spec. Rep. Ser. med. Res. Coun., Lond.*, 1935, No. 201.

Numerous methods have been published which give satisfactory results in the hands of their authors but erratic results when tried elsewhere. In the following method, based on Hurlley's modification of von Fellenberg's process, all possible sources of error have been considered and each detail of procedure rigidly standardised. The original report must be consulted for details of the processes and the preparation of the reagents.

MILK. 40 to 50 ml. of sample is mixed with 4 ml. of 10N potassium hydroxide and set aside for 5 to 6 days in a covered 350 ml. flask stirring occasionally. The mixture is heated on a steam-bath in a glass beaker until homogeneous and then evaporated nearly to dryness on a steam-bath in a nickel dish (free from copper), surface scum being removed after 1 to 2 hours by means of a glass rod which is rinsed and removed. The residue is heated in an oven at 150° to 160° for 1 hour, and then on an electrically heated sand-bath at 400° to 420° for 2 hours, the swollen mass being frequently pierced with a glass rod. It is finally carbonised in an electric muffle at 480° to 500° for 1 hour. The cooled mass is moistened with 20 ml. of water, warmed, filtered through a washed filter (Whatman No. 30), and the residue washed until the total filtrate measures 40 ml. The filter paper is moistened with water in the nickel dish, 0.1 ml. of 10N potassium hydroxide added, the whole stirred thoroughly, and then evaporated to dryness, dried at 150° to 160° for 15 minutes and ignited in the muffle at 480° to 500° for 1 hour. The residue is extracted as before, and the combined filtrates evaporated to dryness, heated in the oven for ½ to 1 hour and then in the muffle for 5 minutes. The product is dissolved in a little water and the evaporation and ignition repeated. The alkaline mass thus obtained is dissolved in 25 ml. of water, digested on a steam-bath, filtered, and the filter washed 2 or 3 times with 3 ml. quantities of N/10 potassium carbonate. The filtrate is evaporated to dryness in a recently ignited porcelain dish, the residue dried for 30 minutes at 150° to 160° and then ignited in the muffle at 480° to 500° for 5 minutes. The alkaline mass is rubbed to a smooth cream with sufficient water (about 2 ml.) to produce almost complete solution (previous to trituration the moistened mass is allowed to stand overnight), 5 ml. of dehydrated alcohol added, followed by two further portions, each of 5 ml., the mass being well rubbed up between each addition and again after all the alcohol has been added until the potassium carbonate

passes into a pasty condition. The alcohol layer is decanted into a recently ignited platinum dish and the residue is re-extracted twice with 5 ml. of dehydrated alcohol, small quantities of water being added if necessary to maintain the pasty condition. The combined alcoholic extracts are treated with 0.15 ml. of N/1 potassium carbonate, and the alcohol is rapidly evaporated by heating the dish on a steam-bath in a current of nitrogen under a cover to prevent ingress of dust, complete removal of the alcohol being effected by heating at 150° to 160° for 5 minutes. The residue is treated with 0.5 ml. of 0.8% potassium nitrate solution and 1 to 2 ml. of water, and rapidly evaporated on the steam-bath, dust being excluded. The residue is dried for 5 minutes at 150° to 160°, then heated in the muffle for 2 minutes only. The product obtained should now be entirely free from carbonaceous matter. It is dissolved in 0.5 ml. of 1% sodium azide and 2 ml. of water, the solution is transferred completely to a titration flask, using 2 quantities, each of 1 ml., of water for rinsing purposes, N/2 sulphuric acid is added until the reaction is acid to methyl orange (as indicated by spotting minute drops on to methyl orange paper), 0.05 ml. more being added than is required for neutrality, and then 0.20 ml. of saturated bromine water. A glass bead is quickly added and the bromine boiled off by means of a micro burner, the flask being agitated gently, until the volume is reduced to about 2 ml. (2½ to 3 minutes) as indicated by a mark etched on the flask. The liquid is then cooled rapidly in water, 0.1 ml. of N/2 sulphuric acid, 0.1 ml. of starch solution and 0.1 ml. of potassium iodide are added, and the mixture titrated with N/500 sodium thiosulphate. Frequent blank determinations must be carried out on a 20% aqueous solution of iodine-free sucrose in order to correct for traces of iodine that may be present in reagents, filter papers or the atmosphere of the laboratory. A correction is applied for differences in the volume of the solution after the bromine boiling process. The weight of iodine found is increased by 0.06 ($V-v$) gamma, where V is the volume after titration in the actual determination, and v the volume in the blank determination.

VEGETABLE MATERIALS. 3 to 5 g. of dry powdered material is mixed with 3 ml. of 10N potassium hydroxide and 30 to 40 ml. of water and heated on a steam-bath for 2 to 3 hours. The process is continued as described above.

BLOOD. 10 ml. of sample is mixed with 20 ml. of water and 1.5 ml. of 10N potassium hydroxide and heated on a steam-bath for 2 to 3 hours. The process is continued as described above. The mass must be repeatedly pierced while being heated on the sand-bath and in the muffle.

OILS AND FATS. Fatty materials including milk powders must be saponified with alcoholic potash before ignition.

DRINKING WATER. 2 to 5 litres of sample is mixed with 4 ml. of 10N potassium carbonate and evaporated in a nickel dish. When nearly dry, 2 ml. of 20% iodine-free sucrose solution is added, and the mixture dried in the oven at 150° to 160°, then heated in the muffle at 480° to 500° for 1 hour. The residue is completely extracted with hot water, and the filtrate treated as described for milk. If much nitrate is present a greater quantity of azide may be required.

MATERIALS CONTAINING SULPHUR. A little iodine-free hydrogen peroxide is added just before the last evaporation in the nickel dish.

FISH PRODUCTS. Ashing is conducted in the presence of iodine-free sucrose to prevent formation of iodate.

SALINE MATERIALS. After the first alcoholic extraction it may be necessary to dissolve the product in water and re-extract with alcohol.

Reagents.

DISTILLED WATER. Re-distilled after being rendered strongly alkaline with potassium hydroxide.

DEHYDRATED ALCOHOL. Dehydrated alcohol is refluxed for 20 minutes over 1% of solid potassium hydroxide. It is then re-distilled in an all-glass apparatus, the first 10 to 20% being rejected and the distillation stopped when 10 to 20% of the original bulk remains. The product has a sp. gr. of not greater than 0.800 and is stored in the dark.

POTASSIUM CARBONATE. Iodine is removed by the following process. 500 g. is dissolved completely in hot water (about 300 ml.) and 0.25 g. of hydrazine sulphate dissolved in a little water is added. The mixture is evaporated in a porcelain dish until it forms a stiff pasty mass on cooling. It is extracted 4 to 5 times with iodine-free alcohol (95 to 96% v/v) using about 200 ml. for each extraction. The residue is dissolved in water, the solution evaporated and the

extraction repeated. Excess of alcohol is removed by suction, and the residue is dried at 150° and ignited in a muffle furnace at 500° for several hours.

20% SUCROSE SOLUTION. The sucrose is freed from iodine by dissolving in the minimum quantity of hot water, adding 10N iodine-free potassium hydroxide (about 2 ml. per 100 g. of sucrose), boiling until partial caramelisation takes place and precipitating with dehydrated alcohol. The sucrose is filtered off and redissolved and reprecipitated several times.

SATURATED BROMINE WATER. This is largely freed from hydrobromic acid by repeatedly washing the bromine with water before preparing the reagent. It is stored in amber-coloured bottles in the dark.

2% POTASSIUM IODIDE SOLUTION. Prepared with recently boiled water. It must be tested at frequent intervals for iodate and free iodine by carrying out a blank test containing 0.1 ml. of N/2 sulphuric acid, 0.1 ml. of the 2% potassium iodide solution and 0.1 ml. of soluble starch solution.

0.5% SOLUBLE STARCH SOLUTION. Soluble starch is prepared as follows:—50 g. of arrowroot starch is mixed with 250 ml. of 2N hydrochloric acid and the mixture allowed to stand for several days at room temperature with occasional stirring. The acid is then decanted and the residue well washed with water by decantation until free from acid and dried on filter paper.

N/500 SODIUM THIOSULPHATE. Prepared with boiled water containing 1% of pure amyl alcohol. It is stored away from direct daylight in a container provided with a soda lime tube and an all-glass siphon. It should be re-standardised at least once a week, using either N/100 potassium bi-iodate or N/500 sulphuric acid with potassium iodide-iodate solution.

C.I.S. Method.

The Second Report of the Commission Internationale des Spécialités includes an agreed method for the determination of iodine in organic substances in which the iodine is converted to iodide by fusion with either potassium hydroxide or a mixture of alkali carbonate and nitrate. The iodide in acid solution is converted to iodate by means of hypochlorite and the free iodine liberated on adding potassium iodide is titrated with sodium thiosulphate.—*Bull. Fed. int. Pharm.*, 1938, 25.

Iodoxylym (B.P. Add. III). $C_8H_5O_5NI_2Na = 493$. Loses on drying in a vacuum desiccator, not more than 1% and then contains 50.5 to 52.5% of I and 9.2 to 9.4% of Na. Assayed for iodine by the method of the *B.P. Add. I* for Thyroxinsodium, and for sodium by ignition and re-ignition with sulphuric acid.

Liquor Diodoni (B.P.C. Supp. III). Determined by the *B.P. Add. I* method for Thyroxinsodium, contains 17.3 to 17.7% w/w of I, corresponding to 34.7 to 35.5% w/w of the diethanolamine salt. Sp. gr., 1.191 to 1.196. Residue, dried at 100° , 35.2 to 35.9%. pH, 6.0 to 8.0.

IPECACUANHA

Ipecacuanha (B.P. Add. III and VI). Contains not less than 2% of the total alkaloids of ipecacuanha, calculated as emetine, of which not less than 55% consists of non-phenolic alkaloids, calculated as emetine and contains not more than 1% of foreign organic matter. Ash, not more than 5%; acid-insoluble ash not more than 2%. Assayed for total alkaloids by maceration and percolation with ether-chloroform (3:1), transferring to acid solution, first with N/1 sulphuric acid and then with portions of N/10 acid and alcohol (3:1), finally extracting with chloroform from ammoniacal solution, determining the alkaloids by titration using methyl red or cochineal indicator; assayed for

non-phenolic alkaloids by extraction of the titration liquid, made alkaline with sodium hydroxide, with ether and, after evaporation, titration of the alkaloids. *Ipecacuanha*, *U.S.P. XII*, should contain not less than 2% of ether-soluble alkaloids of *ipecacuanha*, not more than 5% of its overground stems, or more than 2% of other foreign organic matter. Assayed by maceration with peroxide-free ether and extraction of an aliquot part, using ether for the final extraction of the alkaloids from ammoniacal solution.

Radix Ipecacuanhæ, *P.G. VI*, yields not less than 1.99% of alkaloid, calculated as emetine. *Radix Ipecacuanhæ*, *P. Helv. V*, contains not less than 2% of alkaloid soluble in ether. *Assay*: Shake frequently and vigorously 3 g. of the root in powder with 60 g. of ether and 2.5 ml. of dilute ammonia, for 30 minutes. Pour the supernatant ethereal liquid through cotton wool, evaporate off the ether completely and repeat with a second portion of 5 ml. of ether. Dissolve the residue in 1 ml. of alcohol, add 5 ml. of N/10 hydrochloric acid, 10 drops of methyl red and heat on a water-bath for one minute; dilute with 20 ml. of recently boiled and cooled water and titrate the excess of acid with N/10 sodium hydroxide: each millilitre of N/10 HCl is equivalent to 0.0238 g. of ether-soluble alkaloid.

Paul and Cownley stated the average composition of Rio and Carthagena alkaloids to be: emetine in Rio 72%, in Carthagena 40.5%; cephaeline in Rio 25.9%, Carthagena 56.8%. See also H. R. Jensen, *Pharm. J.*, i/1916, 519.

In the initial maceration of the *B.P.* assay process the powdered drug should be shaken with the ether-chloroform mixture for at least 15 minutes, otherwise percolation to complete extraction is very prolonged.—P. A. W. Self, *Pharm. J.*, i/1933, 244.

Of 37 samples, only 7 failed to comply with *B.P. 1932* requirements for alkaloids. The average content of total alkaloids was 2.31%, and of non-phenolic alkaloids calculated as emetine, was 1.59%.—C. E. Corfield, *Quart. J. Pharm.*, 1934, 603.

***Ipecacuanha Pulverata* (*B.P. Add. VI*).** Adjusted to contain 1.9 to 2.1% of total alkaloids of *ipecacuanha*, calculated as emetine, not less than 55% being non-phenolic alkaloids, calculated as emetine. Ash, not more than 5%; acid-insoluble ash not more than 2%.

***Extractum Ipecacuanhæ Liquidum* (*B.P.*).** Contains 1.9 to 2.1% of total alkaloids of *ipecacuanha*, calculated as emetine. Assayed by extraction of an acid liquid, made ammoniacal, with chloroform, evaporation and titration of the alkaloid. Alcohol content, 75 to 80% *v/v* of ethyl alcohol. *Fluidextractum Ipecacuanhæ*, *U.S.P. XII*, by a maceration process with peroxide-free ether, transference of the alkaloid from an aliquot part to N/1 sulphuric acid with final extraction from ammoniacal solution with peroxide-free ether, and titration of the alkaloid, yields 1.8 to 2.2% *w/v* of ether-soluble alkaloids of *ipecacuanha*.

Tentative methods for the determination of total alkaloids in fluidextract of *ipecacuanha* are described in *Methods of Analysis* (*A.O.A.C.*, 1940, 598).

***Tinctura Ipecacuanhæ* (*B.P.*).** Contains 0.095 to 0.105% *w/v* of total alkaloids of *ipecacuanha*, calculated as emetine. Alcohol content, 20 to 24% *v/v* of ethyl alcohol. Assayed by

cleansing of an acid mixture with chloroform, followed by ammoniacal chloroform extraction. *Tinctura Ipecacuanhæ, N.F. VII*, yields 0.18 to 0.22% *w/v* of ether-soluble alkaloids of ipecacuanha. *Tinctura Ipecacuanhæ I.A.* contains 0.2% of total alkaloids and is prepared with 70% alcohol.

Emetina (B.P.C.). $C_{29}H_{40}O_4N_2 = 480.3$. Loss on drying in a vacuum at atmospheric temperature, not more than 1%. Ash, not more than 0.1%. Complies with the limit test for cephaeline.

Emetinæ Hydrochloridum (B.P.). $C_{29}H_{40}O_4N_2 \cdot 2HCl \cdot 7H_2O = 679.4$. Loss at 100° , 15 to 19%. Ash, not more than 0.1%. By extracting emetine with chloroform, from a solution made alkaline with sodium hydroxide, acidifying the aqueous liquid, adding ammonia, extracting with chloroform and drying at 100° , a limit of cephaeline equivalent to not more than 2% of the alkaloid is included. *Emetinæ Hydrochloridum, U.S.P. XII*, should lose 8 to 14% at 100° . The same limit of cephaeline, but extracted with ether, is allowed. 0.1 g. in 5 ml. of sulphuric acid conforms with a test for carbonisable substances.

An official method for the determination of emetine hydrochloride in tablets is described in *Methods of Analysis (A.O.A.C., 1940, 584)*. The emetine, extracted with ether from alkaline solution, is dissolved in alcohol and titrated with standard acid, using methyl red indicator.

A colorimetric method for the determination of emetine in hypodermic tablets and injections is described. The method depends upon the orange colour produced by the action of hydrogen peroxide on emetine in the presence of hydrochloric acid and can only be used to detect gross errors. Tables are given correlating the value of the yellow component of the colour obtained and the emetine content.—N. L. Allport and N. R. Jones, *Quart. J. Pharm.*, 1942, 244.

Emetinæ et Bismuthi Iodidum (B.P.). Contains 25 to 28% of emetine, $C_{29}H_{40}O_4N_2$, and 18 to 21% of Bi. Loss at 100° , not more than 2%. Assayed for emetine by titration, with N/10 sulphuric acid, of the alkaloid extracted with chloroform from an acid mixture made ammoniacal; and for bismuth by precipitation with ammonium phosphate from the ammoniacal liquids just neutralised with hydrochloric acid, finally weighing the ignited residue.

JALAPA

Jalapæ (B.P.). Should contain not more than 2% of foreign organic matter, and not less than 9% of resin. Assayed by the *B.P.* method by continuous extraction of 20 g. with 90% alcohol, evaporation of the solvent, washing the residue with four quantities of 10 ml. of hot water, and drying at 100° . *Jalapæ, N.F. VII*, should yield not less than 9% of total resins of jalap; assayed by the *N.F. VII* method for *Ipomœa*; acid-insoluble ash, not more than 0.5%. Brazilian jalap, which contains a much higher percentage of a similar resin, is not included in the *B.P.*

Tubera Jalapæ, P.G. VI, contains not less than 10% of resin. *Tuber Jalapæ, P. Helv. V*, contains not less than 10% of resin which should yield not more than 3% to ether.

The following test for purity is recommended by the Sub-Committee on the Assay of Galenicals of the Committee on Pharmaceutical Chemistry:—Weigh 0.5 g. of the residue obtained in the assay, finely powdered, in a tared dry 100-ml. flask, add 25 ml. of anæsthetic ether, shake vigorously for fifteen minutes, and allow to stand overnight; pour off the ether solution into a dry flask, wash the residue with 10 ml. of anæsthetic ether, and dry at 100°; the weight of the residue is not less than 0.425 g. (limit of ether-soluble resins).—(*British Pharmacopœia Commission Report*, No. 11, May 1939.)

Jalapæ Pulverata (B.P.). Adjusted with powdered exhausted jalap or with lactose to contain 9 to 11% of resin.

Jalapæ Resina (B.P.C.). The dried substance contains not less than 85% of ether-insoluble resins. Loss at 100°, not more than 5%. Ash, not more than 0.5%. Water-soluble matter, not more than 2%. Assayed by shaking vigorously with 50 parts of freshly distilled ether, standing and evaporating, and drying at 100° the decanted ether and washings. Resina Jalapæ, *N.F. VII*, should yield not more than 32% of chloroform-soluble matter, dried at 100°, and not more than 12% of ether-soluble resins; loss at 100°, not more than 4%, Résine de Jalap, *Fr. Cx.* 1937, yields not more than 3% insoluble in chloroform and not more than 12% insoluble in ether; acid value 26 to 28; saponification value, 234 to 244. Resina Jalapæ, *P. Helv. V*, yields not more than 3% to ether, when 1 g. in powder is shaken frequently during 6 hours with 10 g. of ether.

Ipomœa (B.P.). Ipomœa or Orizaba jalap root (Mexican scammony root) is the dried root of *Ipomœa orizabensis* (Pellet.) Levanos. The resin extracted with 90% alcohol and washed has the properties of Scammonizæ Resina.

Ipomœa, *N.F. VII*, yields not less than 15% of total resins; acid-insoluble ash, not more than 3%. Assayed by digestion on a steam-bath for 30 minutes and percolation with alcohol and water (9 : 1), extraction of an aliquot part with chloroform and hydrochloric acid (1%) and chloroform and alcohol (2 : 1), finally drying the residue from the evaporated chloroform alcohol at 100°.

Scammonizæ Resina (B.P.). Loss at 100°, not more than 5%. Ash, not more than 0.5%. Water-soluble matter, not more than 1%. Contains not more than 40% of ether-insoluble resins, and the ether-soluble resins have an acid value not exceeding 30. Resina Ipomœæ, *N.F. VII*, leaves not more than 0.5% of ash, and loses not more than 4% at 100°; alcohol-insoluble residue, not more than 0.5%.

Résine de Scammonée, *Fr. Cx.* 1937, has an acid value of 15 to 33, saponification value of 180 to 185; α_D of 4% solution in alcohol (95%), decolorised with charcoal, -16° to -24° , allowing for weight of resin soluble. Résine de Scammonée du Mexique leaves not more than 40% insoluble in ether; acid value, 14 to 28; saponification value, 180 to 229.

Radix Scammonizæ, *P. Helv. V*, is described as the dry root of *Convolvulus Scammonia* L., containing not less than 18% of resin; neither scammony nor ipomœa resin is official. Scammonée d'Alep, *Fr. Cx.* 1937, on mixing with an equal weight of sand, shaking during 12 hours and washing with ether, yields not less than 60% of resin.

Resins from convolvulaceous drugs may be identified by shaking 0.05 g. of powdered resin with acetone 5 ml., filtering, placing 2 ml. of the filtrate and 10 ml. of hydrochloric acid in a test-tube and immersing in a boiling water-bath for ten to fifteen minutes, when a purplish-red colour forms, turning magenta on dilution with one or two volumes of water. Positive reactions were obtained with jalap and scammony and with 2 ml. of a 10% acetone tincture of jalap tuber, ipomœa root and turpeth root.—E. J. Schorn, *Pharm. J.*, i/1941, 206.

LIGAMENTA

Ligamentum Crispi (B.P.C.). Crêpe bandage when fully extended should measure not less than twice the length of the

unstretched bandage; after being fully extended for one minute, it returns to not more than two-thirds the fully extended length. Contains not less than 33.3% of wool, all in the warp. A 3-inch bandage contains in the warp not less than 47 cotton threads and 94 wool threads with 2 two-fold binding threads at each edge. There are one two-fold cotton thread (right twist), 2 wool threads, one two-fold cotton thread (reverse twist), 2 wool threads, in the warp threads. Count of the wool, not coarser than 25's and not finer than 30's (worsted count). The warp threads are made of two-fold cotton yarn with a finished count, after doubling, not finer than two-fold 20's and containing not fewer than 54 folded turns per inch. Weft should consist of 20's count cotton threads, numbering not less than 25 per inch when fully stretched. Weight of 3 inch wide fully stretched bandage, not less than 617 grains per 5 yards.

Ligamentum Domettæ (B.P.C.). Domette bandage contains not less than 66.6% of wool. Foreign matter, not more than 2%; weight of 2 inch by 6 yards bandage, not less than 440 gr. Minimum average number of threads per inch, 40 in the warp and 22 in the weft, and the total number per sq. inch, 65.

Ligamentum Elasticum Adhesivum (B.P.C.). A fully stretched elastic adhesive bandage of 3 inches by 5 yards contains not less than 2 oz. of rubber adhesive compound, and after removal of this and other foreign matter, and correcting for natural moisture regain, weighs not less than 1.75 oz. When released the fully stretched bandage returns to not more than 80% of its length. Warp threads not less than 44 per inch and made of two-fold cotton yarn with a finished count, after doubling, not finer than two-fold 26's and containing not fewer than 44 folded turns per inch, and woven 2 ends right twist and 2 ends reverse twist. Weft threads not less than 20 per inch when fully stretched, and of cotton yarn not finer than 8.5's.

Ligamentum Lanulæ (B.P.C.). Flannel bandage must contain not more than 3% of foreign matter. Weight, not less than 900 gr. per 2 inches by 6 yards. Minimum average threads per inch, 26 in the warp and 28 in the weft.

Ligamentum Linæ (B.P.C.). Bleached calico bandage contains not more than 1.5% of foreign matter. Weight, not less than 210 gr. per 2 inches by 4 yards. Minimum average threads per inch, 67 in the warp and 58 in the weft.

Ligamentum Linæ Crudæ (B.P.C.). Unbleached calico bandage contains not more than 10% of foreign matter. Weight, not less than 250 gr. per 2 inches by 4 yards. Minimum average threads per inch, 65 in the warp and 60 in the weft.

Ligamentum Pastæ Zinci (B.P.C.). The warp of zinc paste bandage should be of cotton yarn of a count not finer than 40's and not heavier than 30's, and from 30 to 35 threads per inch; the weft, of cotton yarn, 26's to 16's and 20 to 25 threads per inch.

Ligamentum Sindonis (B.P.C.). Muslin bandage contains not more than 1.5% of foreign matter. Weight per 2.5 inches by 6 yards, not less than 190 gr. Minimum average threads per inch, 48 in the warp and 30 in the weft.

Ligamentum Textum Apertum (B.P.C.). Open-weave bandage contains not more than 1.5% of foreign matter. Weight per 2 inches by 4 yards, not less than 200 gr. Minimum average threads per inch, 43 in the warp and 27 in the weft. Bleached count of the warp yarn, 40's to 33's.

LINUM

Linum (B.P.). Should contain not more than 2% of foreign organic matter. *Linum, U.S.P. XII*, contains not more than 2%

of other seeds or foreign organic matter. Non-volatile ether-soluble extractive, not less than 30%. Unsaponifiable matter of the non-volatile ether-soluble extractive, not more than 2%.

Linum Contusum (B.P.). Ash not more than 5%. Should yield not less than 30% of fixed oil having the characters and properties of *Oleum Lini*, B.P. '32, when continuously extracted with ether.

Ispaghula (B.P.C.). Foreign organic matter, not more than 3%. Weight of 100 seeds, 0.17 g. to 0.22 g. Agitated in a 25 ml. stoppered cylinder with 20 ml. of water occasionally during 24 hours and allowed to stand for one hour, 1 g. occupies not less than 10 ml.

Psyllium (B.P.C.). Foreign organic matter, not more than 3%. Weight of 100 seeds, 0.09 g. to 0.13 g. 1 g. agitated with water, as described under Ispaghula, occupies not less than 12 ml. *Plantaginis Semen*, N.F. VII, may be obtained from *Plantago ovata* Forskal, and from *P. Psyllium* Linné and *P. arenaria* Waldstein et Kitaibel; foreign organic matter, not more than 0.5%; ash, not more than 4%; acid-insoluble ash, not more than 1%; 1 g. of seed with water to 20 ml. shaken during 24 hours and stood during 12 hours occupies not less than 14 ml. for *P. Psyllium*, not less than 10 ml. for *P. ovata*, and not less than 8 ml. for *P. arenaria*.

Ispaghula and Psyllium of Commerce. All the seeds present in commercial samples of ispaghula examined are seeds of *P. ovata*. Seed of *P. amplexicaulis* appears to be unobtainable commercially. Commercial samples of "Psyllium" seed sometimes consist of seeds of either *P. arenaria* or *P. lanceolata*, partly or wholly. "Bartung" or "Barhang" consists of seeds of *P. major*. It does not occur as an article of commerce in European countries although used widely in the East.—E. W. Skyrme, *Quart. J. Pharm.*, 1935, 11.

A tabular summary of the microscopical characters of the seeds of psyllium, ispaghula and three other species of *Plantago*.—E. W. Skyrme and T. E. Wallis, *Quart. J. Pharm.*, 1936, 198.

LITHIUM

Lithii Acetylsalicylas (B.P.C.). $\text{CH}_3\text{CO}\cdot\text{OC}_6\text{H}_4\cdot\text{COOLi}$ = 186.0. The lithium is assayed as Li_2SO_4 and should be equivalent to not less than 95% of pure acetylsalicylate.

Lithii Benzoas (B.P.C.). $\text{C}_6\text{H}_5\cdot\text{COOLi}$ = 128.0. Contains not less than 98.5% and is assayed by the B.P. process for sodium benzoate. Lithii Benzoas, N.F. VII, determined on the salt dried at 100°, by titration in ethereal solution with N/2 hydrochloric acid to methyl orange indicator, contains 98.5% of the pure substance; loss at 100°, not more than 3%. Benzoate de Lithium, Fr. Cx. 1937, assayed by titration with N/10 hydrochloric acid to helianthine, removing the liberated benzoic acid with ether, should contain 99%, on the dried substance, and by weight of the extracted benzoic acid should contain not less than 94.41% of benzoic acid.

Lithii Bromidum (B.P.C.). LiBr = 86.86. By titration with excess of silver nitrate and ammonium thiocyanate the dried salt

contains 98% of LiBr. Loss at 160°, not more than 15%. Tests for limit of bromate and iodide are included. Lithii Bromidum, *N.F. VII*, contains 85 to 90% of the anhydrous salt; a limit of "other alkalis," by weighing the chlorides insoluble in amyl alcohol, is included.

Lithii Carbonas (*B.P.C.*). $\text{Li}_2\text{CO}_3=73.88$. Assayed by titration with excess acid and sodium hydroxide, a purity of not less than 98.5% on the dried substance should be indicated. Loss at 100°, not more than 1%. Lithii Carbonas, *N.F. VII*, after drying to constant weight at 100°, contains not less than 99% of Li_2CO_3 ; leaves not more than 0.15% of residue insoluble in dilute acetic acid; a limit of "other alkalis," by weight of the chlorides insoluble in amyl alcohol is included; loss at 100°, not more than 2%.

Lithii Chloridum (*B.P.C.*). $\text{LiCl}=42.40$. Loses, on drying at 100°, not more than 5% of its weight and then contains 99% of LiCl by titration of the chloride.

Lithii Citras (*B.P.C.*). $\text{C}_6\text{H}_5\text{O}_7\text{Li}_3, 4\text{H}_2\text{O}=281.9$. Should contain 98.5% of the pure salt; the assay by weighing the residue of Li_2SO_4 when ignited with sulphuric acid, replaces the *B.P.* '14 estimation of the alkalinity of the ash. The *N.F. VII* salt, determined by titration of the ash with standard sulphuric acid, contains not less than 98.5% of $\text{C}_6\text{H}_5\text{O}_7\text{Li}_3$ after drying at 165°. Loss at 165°, not more than 26%. A test for other alkalis is included—the residue obtained by ignition with hydrochloric acid is extracted with water, the residue on evaporation of this water extract is boiled with amyl alcohol and hydrochloric acid and the insoluble matter dried at 110°.

Lithii Iodidum (*B.P.C.*). $\text{LiI}=133.9$. Loses not more than 10% at 120° and the dried salt contains 99% of LiI. The iodide may be estimated by titration with standard potassium iodate.

Lithii Salicylas (*B.P.C. Supp. IV*). $\text{C}_7\text{H}_5\text{O}_3\text{Li}=144.0$. Dried at 130° it contains not less than 98.5% of $\text{C}_7\text{H}_5\text{O}_3\text{Li}$. Loses not more than 3% of its weight at 130°. Assayed by the *B.P.* method for Sodii Salicylas: about 3 g. dissolved in 50 ml. of water, with 50 ml. of ether and a little bromophenol blue indicator are shaken and titrated with N/2 sulphuric acid until a colour change takes place; the titration of the separated aqueous layer, a 10 ml. water washing of the ethereal layer and a fresh 20 ml. of ether is completed, with constant shaking. Lithii Salicylas, *N.F. VII*, dried to constant weight at 100°, contains not less than 98.5% of $\text{LiC}_7\text{H}_5\text{O}_3$. Determined by titration, in ethereal mixture, with N/2 hydrochloric acid to bromophenol blue indicator, separating the ether and continuing the titration of the aqueous portion after addition of more ether. Salicylate de Lithium, *Fr. Cx.* 1937, assayed similarly to the *B.P.C.* method, contains not less than 99% corresponding to 94.93% of salicylic acid; the salicylic acid content is determined by weight of the precipitate produced with iodine in alkaline solution.

LOBELIA

Lobelia (B.P.). Contains not more than 60% of stems and not more than 2% of foreign organic matter. Acid-insoluble ash, not more than 5%. Lobelia, *N.F. VII*, should contain not more than 10% of stems and 4% of other foreign organic matter, and should leave not more than 5% of acid-insoluble ash. *Herba Lobeliae, P. Helv. V*, when assayed by the process prescribed, yields not less than 0.3% of alkaloids; ash, not more than 13%.

Assay. Introduce 10 g. of the lobelia in No. 60 powder and 10 g. of ignited sand into a pear-shaped separating funnel provided with a plug of cotton wool in the tube below the stopcock. Add 75 ml. of a mixture of 4 vols. of ether and 1 vol. of 95% alcohol (by volume). Shake and set aside for 15 minutes, add 5 ml. of dilute ammonia (10% of NH_3), and shake in a mechanical shaker for 1 hour or by hand for 1 minute at 10-minute intervals during one hour. Allow the liquid to percolate into another separating funnel. When the liquid ceases to flow, pack the drug firmly by means of a button-ended glass rod. Continue the percolation, first with 25 ml. of the ether-alcohol mixture and then with ether, until the alkaloid is completely extracted, as shown by testing in the usual manner. To the percolate add 30 ml. of N sulphuric acid. Shake well and allow to separate. Run off the lower layer into another separator. Repeat the extraction with a mixture of 25 ml. of 0.5 N sulphuric acid with 5 ml. of alcohol (95%). Run off the lower layer and repeat with three further quantities of 20 ml. of the acid-alcohol mixture or until the alkaloids are completely extracted. Wash the mixed acid solutions, first with 10 ml. and then with successive quantities of 5 ml. of chloroform, washing each chloroform solution with the same 20 ml. of 0.5 N sulphuric acid contained in another separator. Reject the chloroform, transfer the acid liquid from the second separator to the first separator, neutralise to litmus with dilute ammonia solution and add a further 5 ml. in excess. Extract the alkaloids by shaking with successive quantities of 10 ml. of chloroform. Combine the chloroform solutions and wash with 3 ml. of distilled water. Filter the chloroform solution through a 7-cm. filter paper into a flask. Wash the filter thoroughly with more chloroform and collect the washings in the flask. Distil the chloroform from a water-bath until about 2 ml. remain. Add 2 ml. of absolute alcohol and continue the evaporation on the water-bath, using a gentle air-blast to complete the process. Repeat with two further lots of absolute alcohol to ensure dehydration of the residue. Heat the residue for 1 hour at 80°. Add to the residue 2 ml. of alcohol (95%) and warm until dissolved. Add 10 ml. of 0.02 N sulphuric acid. Cool and titrate with 0.02 N sodium hydroxide or sodium borate solution, using methyl red as indicator. One ml. of 0.02 N sulphuric acid is equivalent to 0.00674 g. of the alkaloids of lobelia calculated as lobeline.—per *Analyst*, 1939, 581.

Hydrochloric acid cannot replace sulphuric acid in the assay because lobeline hydrochloride is extracted from aqueous solution by chloroform. Thorough dehydration of the alkaloidal extractive before heating it is necessary to avoid hydrolysis and production of acetophenone.—W. A. N. Markwell, *Pharm. J.*, i/1936, 617.

Assays of lobelia and its preparations are examined, and recommended processes are described for the drug and tinctures.—H. A. Caulkin, *Quart. J. Pharm.*, 1939, 438.

Lobélie Enflée, Fr. Cx. 1937, contains not less than 0.375% of total alkaloids:—Moisten the equivalent of 12.5 g. of drug dried at 100° with 40 g. ammoniacal alcohol (90% with 10% ammonia solution), after 3 hours add 360 g. ether and shake frequently during 3 to 4 hours; filter and reduce by distillation the solvent from 320 g. (10 g. powder) to 10 g.; transfer to a separator with 3 portions of 150 ml. of ether and shake with 15 ml. N/1 hydrochloric acid, completely extract with further quantities of 5 ml. (test with silico-tungstic acid). Allow the ether to evaporate from the mixed acid liquids, add 10 ml. of silico-tungstic acid solution (5%) and after 12 hours, collect the precipitate, wash with N/1 hydrochloric acid until the washings do not precipitate quinine hydrochloride solution (1%) and ignite. 1 g. residue = 0.415 g. total alkaloids.

Lobelinum hydrochloridum, $C_{21}H_{27}O_2N.HCl$, is official in the *P.G. VI*. Lobelinum hydrochloricum, *P. Helv. V*, dried over sulphuric acid, contains not more than 1% of moisture; 0.4 g. in 20 ml. of water at 20° in a 200 mm. tube has an optical rotation of -2.23° to -2.33° ; the separated alkaloid, after drying over sulphuric acid for 24 hours, melts at a temperature not below 116° . Titration with sodium hydroxide to phenolphthalein should show not less than 99.3% of $C_{21}H_{27}O_2N.HCl$.

MAGNESIUM

Magnesii Carbonas Levis (B.P.). Residue on ignition, 42 to 45%; calcium limit, 2% as CaO. Magnesii Carbonas, *U.S.P. XII*, by titration with excess of N/1 sulphuric acid and N/1 sodium hydroxide to methyl orange, contains the equivalent of 40 to 43.5% of MgO; a calcium limit equivalent to not more than 0.6% of CaO is included. Magnesii Carbonas Ponderosus, *B.P.*, leaves a residue on ignition of 42 to 45% and has a limit of 0.7% of CaO. Magnesium subcarbonicum, *P. Helv. V*, yields on ignition from 39.0 to 44.1% of residue and the residue contains not less than 99% of MgO.

Liquor Magnesii Bicarbonatis (B.P.). Should contain not less than 2.5% *w/v* of $Mg(HCO_3)_2$. Assayed by titration of total alkalinity to methyl orange with subtraction of the alkali limit titration obtained by boiling the ignited residue with water, filtering and titrating.

Magnesii Hydroxidum (B.P.C.). $Mg(OH)_2 = 58.34$. Residue on ignition, 67 to 70%.

Mistura Magnesii Hydroxidi (B.P.). Contains the equivalent of 7.75 to 8.75% *w/v* of $Mg(OH)_2$. Magma Magnesiae, *U.S.P. XII*, contains 7 to 8.5% of $Mg(OH)_2$. It may contain 0.1% citric acid. In the test for heavy metals, 5 ml. is dissolved in 6 ml. of dilute hydrochloric acid and the solution evaporated to dryness. The residue is dissolved in 20 ml. of distilled water, 2 ml. of dilute acetic acid added and the test completed in the usual manner.

Magnesii Oxidum Leve (B.P.). $MgO = 40.32$. Loss on ignition, not more than 5%; calcium limit, 5% as CaO. Magnesii Oxidum, *U.S.P. XII*, loses not more than 10% on ignition, and then contains not less than 96% MgO, by titration of alkalinity to methyl orange less the calcium determined as for the carbonate; CaO limit, 1.5%. Magnesii Oxidum Ponderosum, *B.P.*, loses not more than 5% on ignition and has a calcium limit of 1.75% of CaO. The *U.S.P. XII* substance loses not more than 10% on ignition, and then contains not less than 96% of MgO. Magnesium oxydatum, *P. Helv. V*, loses not more than 15% of its weight on ignition, and the ignited substance contains not less than 99% of MgO.

Magnesii Trisilicas (B.P. Add. IV and VI). Loses 20 to 30% on ignition at dull red heat and then contains magnesium equivalent to 30.0 to 31.5% of MgO and silicon equivalent to 66 to 69.5% of SiO_2 . Assayed for silicon by digestion with hydro-

chloric acid for 3 hours, heating at 105° for 2 hours, further digestion with hydrochloric acid, and water, and after separation of the silica and further recovery from the filtrate, ignition to constant weight and subtraction of the portion non-volatile with sulphuric and hydrofluoric acids; magnesium is precipitated from the filtrates as magnesium ammonium phosphate, finally weighing as pyrophosphate. A test for neutralising power to N/20 hydrochloric acid, when digested at 37° for 3 hours is included; the equivalent of 1 g. of the ignited substance neutralises not less than 250 ml. of N/20 acid. The *U.S.P. XII* substance contains not less than 20% of MgO, not less than 45% of SiO_2 and loses on ignition not more than 34%. The ratio of the percentage of SiO_2 to the percentage of MgO lies between 2.10 and 2.30. A test for adsorptive power using methylene blue solution and a limit test for heavy metals are included. 1 g. of the anhydrous substance when digested at 37° for 4 hours with occasional shaking, is equivalent to not less than 140 and not more than 160 ml. of N/10 hydrochloric acid.

Tabellæ Magnesii Trisilicatis (*U.S.P. XII*). Not less than 20 tablets, when reduced to a fine powder, completely pass through a 200 mesh sieve and comply with tests for free alkali, acid consuming capacity and adsorption of methylene blue. Tested for free alkali by boiling with water, filtering, cooling and titrating with N/10 hydrochloric acid, using phenolphthalein indicator. Acid consuming capacity determined by digestion of a weighed quantity of powdered tablets with N/10 hydrochloric acid at 37° with occasional shaking throughout 4 hours, the mixture being left undisturbed for the last 15 minutes, and finally titrating an aliquot of the supernatant liquid with N/10 sodium hydroxide using methyl orange indicator. The volume of N/10 acid consumed corresponds to 85 to 110 ml. per g. of magnesium trisilicate taken. Tested for adsorption of methylene blue by shaking a weighed quantity of powdered tablets with 0.1% aqueous methylene blue solution continuously for 5 minutes and then intermittently for 1 hour, filtering and comparing the colour of the filtrate with a specified dilution of the methylene blue solution.

Peroxyde de Magnésium, Fr. Cx. 1937, by titration with N/10 potassium permanganate contains not less than 25% of MgO_2 .

Magnesia Mixture. Solution of Magnesium Ammonio-Sulphate. Dissolve magnesium sulphate 20, ammonium chloride 40, in water 160, add ammonia solution 84. Allow to deposit in a stoppered bottle before use. Employed for the gravimetric determination of phosphates. Ammonium magnesium phosphate is precipitated and converted by incineration into magnesium pyrophosphate. $\text{Mg}_2\text{P}_2\text{O}_7$.

Detection of Magnesium. Magnesium may be detected by means of *o-p*-dihydroxyazo-*p*-nitrobenzene (*syn.* Magneson; *o-p*-dihydroxybenzeneazo-*p*-nitrobenzene; *p*-nitrobenzeneazoresorcinol), $\text{NO}_2\text{C}_6\text{H}_4\text{N}:\text{NC}_6\text{H}_3(\text{OH})_2$. The reagent is used as a 0.1% solution in 1% sodium hydroxide solution; it keeps for several months, but should not be used if more than a year old. The test solution is freed from other metallic radicles except the alkali metals by the ordinary group reactions. Ammonium compounds are then removed by evaporating to dryness and igniting. The residue is dissolved in 10 ml. of water acidified with hydrochloric acid, and 1 drop of the reagent is added followed by

10 ml. of sodium hydroxide solution. A blue colour or precipitate visible after about 5 minutes denotes the presence of Mg.

Determination of Magnesium. Magnesium can be determined by means of 8-hydroxyquinoline (*syn.* Oxine), $C_9H_7N.OH$. The reagent is used either as a 5% solution in alcohol or as a 2% solution in 2N acetic acid. The test solution, containing about 0.02 g. of magnesium in 50 ml., is made alkaline with ammonia, sufficient ammonium chloride being added to prevent precipitation, and heated nearly to the boiling-point. The alcoholic reagent solution is then added until the liquid becomes yellow in colour, when a compound of the composition, $Mg(C_8H_6ON)_2.4H_2O$, separates. The precipitate is collected, washed with hot faintly ammoniacal water and dried at 100° to 105° , at which temperature $2H_2O$ is lost. Each gramme of residue is equivalent to 0.0698 g. of Mg. Alternatively, the precipitate may be dissolved in 5N hydrochloric acid, and the filter washed with sufficient water to yield 2N acid. The solution is titrated to a yellow colour with N/5 bromine solution, using methyl red as indicator. Potassium iodide is added and the liberated iodine titrated with N/10 sodium thiosulphate. Each millilitre of N/5 bromine is equivalent to 0.000608 g. of Mg. Direct titration with N/5 bromine is impracticable because of the indefinite end-point. The procedure must be modified in the presence of an excess of an alkaline earth metal.

Magnesium forms with oxine a dihydrate, $Mg(C_8H_6ON)_2.2H_2O$, which is reasonably constant in weight at 110° after $2\frac{1}{2}$ hours, and at 98° after drying for a considerably longer period. Drying this precipitate in a steam-oven for 1 hour before weighing as the hydrate would, therefore, give rise to an appreciable error. It is possible in some instances that a small proportion of 8-hydroxyquinoline is precipitated with the oxinate, and that this is gradually volatilised during the drying process, at a rate dependent on temperature and time. The anhydrous magnesium complex is only obtained after heating the dihydrate at 160° . Hence the most reliable results from a gravimetric determination of magnesium as oxinate are likely to be obtained by drying the precipitate to the anhydrous form at 160° , and errors attributed to other causes may frequently be connected with inadequate drying.—R. C. Chirnside, C. F. Pritchard and H. P. Rooksby, *Analyst*, 1941, 399.

In the presence of ammonium citrate, manganese may be precipitated with oxine from a slightly acid solution containing magnesium without co-precipitation of the magnesium. Upon adding excess of ammonia to the filtrate, previously heated to 70° , and allowing to stand at room temperature for 2 hours complete precipitation of 0.01 g. of magnesium is effected. Solutions containing less than 0.01 g. of Mg should be allowed to stand overnight before filtration. 0.005 g. in 400 ml. of solution was completely precipitated by this means. If the concentration of Mg is smaller, the solution should be concentrated before precipitation. Free oxine in the precipitate may be removed by washing with a little warm alcohol, as well as with hot water. Calcium is co-precipitated only to a small extent in the presence of ammonium citrate, and may be removed as oxalate before precipitation of the magnesium-oxine complex. Alternatively the magnesium complex may be dissolved in dilute hydrochloric acid and reprecipitated. Phosphoric acid does not interfere with the precipitation of magnesium with oxine. The method has been used for the determination of Mg. in compound syrup of glycerophosphates.—G. J. W. Ferrey, *Quart. J. Pharm.*, 1942, 258.

MALTUM

Extractum Malti (*B.P. Add. V*). Determined by the Kjeldahl method, it contains nitrogen equivalent to not less than 4.5% w/w of protein. Sp. gr., 1.40 to 1.42. n_{D20° , 1.4892 to 1.4976. Extractum Malti, *U.S.P. XII*, should be capable of converting *not* less than five times its weight of dried starch into water-soluble sugars; assayed by testing for remaining starch, with N/10 iodine, the resultant solution from a digestion of purified potato starch (equivalent to 5 quantities of dried starch) at 40° for thirty minutes.

Extractum Malti cum Oleo Morrhuae (B.P.). Contains 10% *w/w*, equivalent approximately to 15% *v/v*, of cod-liver oil. For methods of determining the proportion of oil in malt and cod-liver oil, see the notes under *Oleum Morrhuae*.

For the determination of oil in *Extractum Malti cum Oleo Morrhuae*, see p. 286.

Extractum Malti cum Vitaminis (B.P.C.). Each fluid drachm contains approximately 3000 units of vitamin A and approximately 225 units of vitamin D.

Methods of Analysis for All-English Malt Extracts. S.R. & O., 1933, No. 540.

(a) Diastatic Activity (or Lintner Value)

SOLUBLE STARCH. Digest purified potato starch with dilute hydrochloric acid of sp. gr. 1.04 (in the proportion of 1 lb. of starch to 1 litre of dilute acid) at a temperature not exceeding 20° for 7 days, well shaking daily. Thoroughly wash the starch by decantation, first with tap water until washings react only faintly acid, then four times with distilled water. Weigh about 20 g. of the sludge, dissolve in 200 ml. of boiling distilled water and neutralise with N/10 sodium hydroxide solution, using 2 or 3 drops of alizarin cream as indicator. Add to remaining weighed starch sludge the calculated amount of sodium hydroxide solution just to neutralise its acidity, shake thoroughly and set aside for 12 hours. Wash by decantation three times with distilled water, collect the soluble starch on a paper in a Buchner funnel and drain by suction. Transfer to new unglazed porous plates and dry at moderate temperature (40° to 45°). When moisture content is reduced to about 15%, grind the soluble starch in a porcelain mortar and rub through a fine hair sieve.

SOLUBLE STARCH SOLUTION. Rub 20 g. of soluble starch into a cream with water and pour into about 700 ml. of boiling water. Bring to the boil, heat for a further 2 minutes, then cool to about 20°, shaking to prevent formation of skin. Add 20 ml. of acetate buffer solution (see below) and dilute to 1 litre with water. (10 ml. of this solution should not reduce 0.1 ml. of Fehling's solution). Fresh soluble starch solution should be made for each day's determination.

ACETATE BUFFER SOLUTION. One litre to contain 68 g. of sodium acetate ($\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$) and 500 ml. of N/1 acetic acid.

MALT EXTRACT (SYRUP) SOLUTION. 5% solution. Weigh 10 g. of extract and break down with cold water. Heat must not be used to assist in weighing or bringing into solution. Transfer the solution to a 200 ml. graduated flask, dilute to the mark at 15° and shake well. Weaker solutions, also made up at 15°, are prepared from this. These solutions must not be filtered, and are used as soon as possible for starch conversion.

MALT EXTRACT (FLOUR SOLUTION). 5% solution. Weigh 10 g. of flour into a beaker, add 200 ml. of water at 15° and thoroughly stir. Cover and digest for 3 hours in a water-bath at 21°, stirring at intervals of half an hour. Three hours after, filter through filter paper. Reject the first 25 ml. of filtrate, and if the remainder of the filtrate is not quite bright re-filter. This solution, or weaker solutions prepared from it, is used as soon as possible for starch conversion.

METHOD OF STARCH CONVERSION. Measure 100 ml. of soluble starch solution into a 200 ml. graduated flask and immerse, suitably supported, in a water-bath maintained at 21°. Place a standardised thermometer in the flask, and when the contents have reached 21° add, by means of a narrow-bore pipette (N.P.L. standard), a definite volume (which should not normally exceed 10 ml.), measured at 15°, of the malt extract solution and mix well. (The volume needed will depend upon the diastatic activity of the extract and will be about

80

ml. of 5% solution or correspondingly larger
DIASTATIC ACTIVITY OF THE SAMPLE
volumes of 2½% or 1% solution.) Maintain the contents of the flask at 21° for exactly one hour. Then add 20 ml. of N/10 sodium hydroxide solution and mix immediately, care being taken to wash down the thermometer and also to allow

the alkali to flow over the inner surface of the neck of the flask. Cool the solution to 15°, dilute to 200 ml. with water and shake well. This solution is referred to in the method of titration as *the conversion solution*.

METHOD OF TITRATION. Measure into a 200 ml. round-bottomed flask 5 ml. of Fehling's solution (see later), and heat over a naked flame with continuous rotation of flask until solution boils. Run from a burette into the boiling liquid 5 ml. of the conversion solution, and subsequently further quantities. After each addition boil the liquid, the flask being continuously rotated. When the blue colour of the copper solution has nearly disappeared add 0.2 ml. of 1% aqueous solution of methylene blue. Continue the titration with small quantities of the conversion solution, drop by drop, until the blue colour just disappears. (Notes.—The indicator is not added until the end-point is nearly reached since the final change is very rapid. The complete decolorisation of the methylene blue is indicated by the whole reaction liquid, in which the precipitated cuprous oxide is continually being churned up, becoming bright red or orange in colour. To ensure that the end-point has been reached hold the flask against a sheet of white paper, and if the indicator is completely decolorised there will be no blue tint at the edge of the liquid. The boiling process must be sufficiently continuous to prevent air obtaining access to the flask and so causing oxidation of the indicator with reappearance of the blue colour.)

If the volume of the conversion solution used to reduce 5 ml. of Fehling's solution is less than 20 ml. or more than 25 ml., the conversion must be repeated, using less or greater quantities of the malt extract solution, to obtain a titration between these limits. If the extract solutions become aerated or subjected to warm conditions, re-weigh and carry out the dilutions again.

A first titration to obtain approximate results is to be followed by a second, and third if necessary, to establish the end-point accurately. A confirmatory titration should be carried out in every case.

FEHLING'S SOLUTION. Measure into a dry flask equal quantities of the solutions Nos. 1 and 2, and mix.

Solution No. 1. One litre to contain 69.28 g. of crystallised copper sulphate, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$.

Solution No. 2. One litre to contain 346 g. of Rochelle salt and 150 g. of sodium hydroxide.

Freshly mix for each day's determination.

Checked against 0.1% standard invert sugar solution by the method of titration described above, 5 ml. of Fehling's solution corresponds to 0.02533 g. of invert sugar.

Method of Calculating Diastatic Activity (Lintner Value). Express the result according to the following formulæ:—

(a) In the case of flour, diastatic activity (or Lintner value)

$$= \frac{1000}{X \times Y}$$

(b) In the case of syrup, diastatic activity (or Lintner value)

$$= \frac{1000}{X \times Y} \text{ minus } 9.$$

Where X = number of ml. of 5% malt extract solution in 100 ml. of the conversion solution.

Y = number of ml. of conversion solution required to reduce 5 ml. of Fehling's solution.

And 9 = a constant denoting the assumed equivalent of the reducing sugars present in the malt extract (syrup) used in making the determination.

The British Pharmacopoeia Commission Report, No. 11, May 1939, recommends the above method to be used as a limit test of diastatic value for extract of malt. 5 ml. of solution of potassium-cupric tartrate should require not more than 20 ml. of the conversion solution produced when 5 ml. of a 2% solution of extract of malt is treated in the foregoing manner.

(b) **FIBRE CONTENT.** Extract 2 to 3 g. with petroleum spirit, b.p., 40° to 60°, in an extraction apparatus, or at least three times by stirring, settling and decantation, and transfer the dry residue to a conical 1000 ml. flask. The material must not be further ground during extraction. A volume of 200 ml. of

a solution containing 1.25 g. of sulphuric acid (H_2SO_4) per 100 ml. measured at ordinary temperature and brought to boiling point is added and heated. The contents of the flask must boil within one minute and the boiling throughout must be gentle, and continuous for exactly 30 minutes, the original volume being maintained. Rotate the flask every few minutes. After 30 minutes remove the flask and pour contents into the shallow layer of hot water remaining in a funnel fitted with a pump-plate, or into the similar layer remaining in a Buchner funnel. Prepare the funnel by cutting a piece of cotton cloth or filter paper to cover the holes, so as to serve as a support for a disc of ordinary filter paper; pour boiling water into the funnel and allow to remain until funnel is hot, when suction is applied. Discard the experiment if the time of filtration of the bulk of the 200 ml. exceeds 10 minutes. Wash the residues with boiling water until the washings are free from acid. Then wash the residue from the filter paper back into the flask with a volume of 200 ml. of a solution of sodium hydroxide, containing 1.25 g. of sodium hydroxide ($NaOH$) per 100 ml., free from sodium carbonate, measured at ordinary temperature, and brought to boiling point. Boil the contents of the flask for exactly 30 minutes, the precautions given for the treatment with acid being observed. At the end of 30 minutes, remove the flask and filter its contents through an ordinary filter paper. Wash the residue collected in the filter paper with boiling water, then with solution of 1% hydrochloric acid and again with boiling water until free from acid. Wash the residue twice with 95% alcohol, and three times with ether. Transfer the residue to a dried weighed ashless filter paper, dried at about 100° in an oven and weighed in a weighing bottle until constant in weight. Determine the ash of the paper and contents by incineration at a dull red heat. Subtract the weight of ash from the increase of weight found on the paper and report the difference as fibre.

ASH CONTENT. Ascertain the ash content by heating a measured quantity of the substance in a muffle furnace at such a temperature that the ash does not fuse.

PROTEIN CONTENT. Ascertain the amount of soluble protein by multiplying the amount of nitrogen present (other than ammoniacal or nitric nitrogen, if any) by 6.25.

MALT EXTRACTS PRODUCED FROM BARLEY GROWN IN ENGLAND AND WALES: GRADE DESIGNATIONS AND DEFINITIONS

(S.R. & O., 1933, No. 540)

(a) *Pharmaceutical Malt Extract*

| Grade Designation | Definition of Quality † |
|--|--|
| All-English (Pharmaceutical) Malt Extract or alternatively* National Mark (Pharmaceutical) Malt Extract. | <p><i>General.</i> The Extract shall be prepared from sound, clean, malted grain by digestion with water at a suitable temperature and by evaporation of the strained liquid under reduced pressure at a temperature not exceeding 55° until an amber or yellowish-brown viscous product is obtained having the characteristic agreeable odour and sweet taste. The product shall be miscible with water in all proportions, forming a translucent solution.</p> <p><i>Special.</i> The protein content shall be not less than 4.5% of the total weight. The arsenic content shall not exceed 1.4 parts per million. The sp. gr. at 15.5° shall be from 1.40 to 1.42, and the refractive index at 20° from 1.4892 to 1.4976.</p> |

*The alternative "National Mark" may only be used in connection with pharmaceutical malt extract to which the grade designation mark as set out in the Fourth Schedule has been lawfully applied in accordance with the Agricultural Produce (Grading and Marking) (General) Regulations, 1928. (S.R. & O., 1928; No. 674.)

†Extract of Malt as defined in the *British Pharmacopœia*, 1932, would conform with these requirements.

(b) *Bakers' and Veterinary Malt Extracts*

| Grade Designation | Definition of Quality | |
|--|--|---|
| | Particular Characteristics | Common Characteristics |
| All-English (Bakers') Malt Extract (White Bread) | Diastatic activity shall be not less than 40 Lintner value | <i>General.</i> The product in each case shall be the water-soluble extract derived from commercially sound, clean malted grain. |
| All-English (Bakers') Malt Extract (Brown Bread) | None | <i>Special.</i> The specific gravity at 15.50° shall be not less than 1.4 and the soluble protein-content not less than 4.5% of the total weight. |
| All-English (Veterinary) Malt Extract | None | |

MALT FLOURS PRODUCED FROM BARLEY AND/OR WHEAT GROWN IN ENGLAND AND WALES: GRADE DESIGNATIONS AND DEFINITIONS

| Grade Designation | Special Characteristics | |
|--------------------------------------|--|--|
| | Special Characteristics | Common Characteristics |
| All-English Malt Flour (White Bread) | Diastatic activity shall be not less than 40 Lintner value | <i>General.</i> The flour shall be the pure ground product of cleaned malted grain and be sound, free from taint or objectionable flavour and of good keeping quality. |
| All-English Malt Flour (Brown Bread) | None | <i>Special.</i> The maximum content of water shall be 10%, of ash 1.3%, and of fibre 2.5% of the total weight. |

MENTHA PIPERITA

Mentha Piperita (B.P.C.). Should contain not more than 2% of foreign organic matter, and yield not more than 2% of acid-insoluble ash. *Mentha Piperita*, U.S.P. XII, contains not more than 2% of stems more than 3 mm. in diameter, or other foreign organic matter.

Folia Menthae Piperitæ, P.G. VI, yields not less than 0.7% of volatile oil.

Oleum Menthae Piperitæ (B.P. Add. I). Contains 4.0 to 9% w/w of esters, calculated as menthyl acetate, $C_{12}H_{22}O_2$, and not less than 46% w/w of free menthol, $C_{10}H_{20}O$. Sp. gr., 0.902 to 0.915. α_D , -18° to -30° . n_{D20} , 1.460 to 1.470. Soluble in 4 volumes of alcohol (70%, sp. gr. 0.8896 to 0.8901). Assayed for ester content by boiling the neutralised oil with N/2 alcoholic potash for 1 hour and back titrating with N/2 sulphuric acid, and performing a blank; the acetylated oil obtained by boiling gently for 2 hours with acetic anhydride and anhydrous sodium acetate, washing the product and drying over anhydrous sodium sulphate, is treated as

for the ester determination. The free alcohols are calculated from the ester values of the original and acetylated oils using the B.P. formula. *Oleum Menthae Piperitæ, U.S.P. XII*, contains not less than 5% of esters as menthyl acetate, and not less than 50% of total menthol, free and as esters. Assayed similarly to the B.P. process and an equation for calculation is given.

The Food and Drug Administration of the U.S. Dept. of Agriculture defined oil of peppermint, for food purposes, as the volatile oil obtained from peppermint (*Mentha piperita* L.), containing not less than 50% by weight of menthol.—*S.R.A., F.D., No. 2, Rev. 5, Nov. 1936.*

English and American oils are distilled from *Mentha piperita*. Two varieties are used, black and white; the former gives the larger yield of oil and is almost exclusively used, but the latter gives an oil of finer quality.

Studies of the genus *Mentha*.—Kremers, *Perfum. essent. Oil Rec.*, 1925, 409, and *ibid.*, 1926, 90.

For identity of aldehydes, ketones, etc., in American oil, also American peppermint distillation, see Bulletin No. 1555, U.S. Dept. of Agriculture, which also gives details of types of plants, soil, cultivation and distillation. Tabulated figures for a large number of oils taken over two seasons are given by Lazell (*Perfum. essent. Oil Rec.*, 1928, 193).

Evaluation of menthone in peppermint oil. Determination as the semicarbazone is compared with the oxime method.—Reilley, Moonan and Drumm, *Perfum. essent. Oil Rec.*, 1931, 378.

In the determination of menthol the time of acetylation may be varied within wide limits, but the time of saponification should be not less than 45 minutes and not more than 60 minutes in order to get concordant results.—L. H. Baldinger, *J. Amer. pharm. Ass.*, 1939, 155.

Japanese peppermint oil is obtained from *Mentha arvensis*. Undementholised oil contains about 85% of menthol, dementholised oil about 50%; both varieties are commercial oils. Details of the distillation of peppermint oil in Japan are given in *Perfum. essent. Oil Rec.*, 1931, 241. For the constitution of the high boiling fraction of Japanese oil see Shinosaki and Nagasawa, *Perfum. essent. Oil Rec.*, 1931, 172.

The principal terpenes in the oil are limonene and α -pinene, and the sesquiterpene fraction is mostly caryophyllene.—Duncan and Short, *J. Soc. chem. Ind., Lond.*, 1931, 198 T.

Some difficulty has been experienced in obtaining American oils containing the B.P. limit for free menthol. Japanese oil, from *Mentha arvensis*, is not B.P., and redistilled Japanese mint oils will no longer conform to B.P. tests.—C. T. Bennett, *Pharm. J.*, i/1933, 150.

For details of the production and analytical characters of Franco-Mitcham peppermint oil see *Perfum. essent. Oil Rec.*, 1926, 170, and for Italo-Mitcham oil see *Pharm. J.*, i/1935, 289.

A peppermint oil from WESTERN AUSTRALIA compared favourably with the best American production and closely resembled English distilled oil.—E. J. Parry, *Perfum. essent. Oil Rec.*, 1924, 188.

Determination of Menthone. 2 to 3 g. of oil is added to 20 ml. of a 5% solution of hydroxylamine in alcohol. N/2 alcoholic potash is then added, using methyl orange as indicator and keeping the mixture slightly acid, until finally neutralised. Each ml. of N/2 alkali = 0.077 g. of menthone.—G. Perraud, *per Quart. J. Pharm.*, 1936, 120.

Detection of Japanese Mint Oil in Peppermint Oil. A test depending on the colour produced by furfuraldehyde which is present in Japanese mint oil. 0.1 ml. of oil is mixed in a test tube with 5.0 ml. of a 2% solution of freshly distilled aniline in glacial acetic acid, added from a burette. The reaction mixture is examined in a 1 cm. cell of a Lovibond tintometer after an interval of 10 minutes. The reaction mixture must be protected from bright light. Japanese mint oils show a red value from 4.5 to 7.4 whilst peppermint oils give a value of 1.0 or less.—D. C. Garratt, *Analyst*, 1935, 369. The application of this test to other oils, light camphor oil in rosemary oil and clove oil in bay oil or pimento berry oil.—D. C. Garratt, *Analyst*, 1935, 595.

Mentha Viridis (*U.S.P. XII*). Contains not more than 2% of stems more than 3 mm. in diameter, or other foreign organic matter.

Oleum Menthæ Viridis (*B.P.C.*). By the method of the *B.P.* for carvone by digestion with hydroxylamine hydrochloride and titration of the liberated acid with N/1 alcoholic potash to dimethyl yellow, not less than 42% of carvone is indicated. Sp. gr., 0.920 to 0.940. α_D , -34° to -55° . n_{D20° , 1.483 to 1.490. Soluble in three volumes of alcohol (90%, sp. gr. 0.8334 to 0.8340). Oleum Menthæ Viridis, *U.S.P. XII*, yields by the sodium sulphite absorption not less than 50% by volume of carvone; sp. gr. at 25° , 0.917 to 0.934; α_D at 25° , -48° to -59° ; n_{D20° , 1.4840 to 1.4910.

Oleum Pulegii (*B.P.C.*). Contains not less than 85% *v/v* of pulegone. Sp. gr., 0.930 to 0.960; α_D , $+14^\circ$ to $+28^\circ$; n_{D20° , 1.475 to 1.490. Soluble in three volumes of alcohol (70%, sp. gr. 0.8896 to 0.8901). Assayed by measurement of the oil unabsorbed by sodium sulphite solution and deduction from the original volume.

Menthol (*B.P.*, *B.P. Add. I* and *B.P. Add. IV*). Consists of lævo-menthol (obtained from the volatile oils of various species of *Mentha*), or prepared synthetically, synthetic racemic menthol, or mixed isomeric menthols (any mixture of the stereoisomers of *p*-menthan-3-ol). Complies with tests for neutrality and absence of phenolic substances. Residue on evaporation from an open dish on a water-bath, not more than 0.05%. M.p. of lævo-menthol, 42° to 44° , α_D at 20° of 10% *w/v* solution of lævo-menthol in alcohol (90%), -49° to -50° . Freezing point of racemic menthol, -27° to -28° , rising on stirring to 30° to 32° ; m.p., 32.5° to 34° ; optically inactive. M.p. of mixed isomeric menthols, not below 31.5° . Menthol, *U.S.P. XII*, has a m.p. of 41° to 43° , and complies with tests for wax, paraffin or inorganic substances, and for thymol.

An official method for the determination of menthol is described in *Methods of Analysis* (*A.O.A.C.*, 1940, 599). The menthol is acetylated with acetic anhydride and sodium acetate and a definite amount of the washed and dried acetylated oil refluxed with an excess of potassium hydroxide. On cooling, the excess of alkali is titrated with sulphuric acid, using phenolphthalein as indicator. The percentage of menthol is calculated from the formula: $-A \times 7.808 \div [B - (A \times 0.021)]$, where A is the volume of potassium hydroxide solution used, and B the weight of acetylated oil taken.

MYRISTICA

Myristica (*B.P.*). Nutmegs should consist only of the dried kernels of *Myristica fragrans*. Myristica, *U.S.P. XII*, yields not less than 25% of non-volatile, ether-soluble extractive and not more than 0.5% of acid-insoluble ash.

The Food and Drug Administration of the U.S. Dept. of Agriculture defined nutmeg as the dried seed of *Myristica fragrans* Houtt, deprived of its testa, with or without a coating of lime, containing not less than 25% of non-volatile ether extract, not more than 10% of crude fibre, not more than 5% of total ash nor more than 0.5% of ash insoluble in hydrochloric acid. Macassar nutmeg, Papua nutmeg, male nutmeg, long nutmeg, was defined as the dried seed of *Myristica argentea* Warb., deprived of its testa.—*S.R.A., F.D. No. 2, Rev. 5, Nov. 1936.*

Oleum Myristicæ (B.P.). Sp. gr., 0.880 to 0.925. α_D , $+10^\circ$ to $+30^\circ$; n_{D20° , 1.474 to 1.488. Residue on evaporation in a flat dish on a water-bath, not more than 3%. Soluble in three volumes of alcohol (90%, sp. gr. 0.8334 to 0.8340). The *B.P. Add. I* specifies that the evaporation for determination of non-volatile residue be made from a flat-bottomed dish, 9 cm. diameter and 1.5 cm. in depth. The *U.S.P. XII* oil has a sp. gr. at 25° of 0.880 to 0.910; α_D at 25° , $+10^\circ$ to $+30^\circ$; n_{D20° , 1.4740 to 1.4880; residue on evaporation, not more than 2%.

Oleum Myristicæ Deterpenatum (B.P.C.). Soluble in three volumes of alcohol (80%, sp. gr. 0.8634 to 0.8640). Sp. gr., 1.040 to 1.100; α_D , $+1^\circ$ to $+14^\circ$; n_{D20° , 1.500 to 1.533.

Mace. The Food and Drug Administration of the U.S. Dept. of Agriculture defined mace as the dried arillus of *Myristica fragrans* Houtt., containing not less than 20% nor more than 30% of non-volatile ether extract, not more than 10% of crude fibre, not more than 3% of total ash, and not more than 0.5% of ash insoluble in hydrochloric acid. Macassar mace, Papua mace: the dried arillus of *Myristica argentea* Warb.—*S.R.A., F.D., No. 2, Rev. 5, Nov. 1936.*

The Nutmeg Industry. The principal sources of supply are the Dutch East Indies and Granada, the former exporting about three times as much as the latter. East Indian nutmegs are "round," i.e., nearly spherical. Granada nutmegs are mixed "round" and "long," i.e., length exceeding diameter by $1\frac{1}{2}$ to 2 times. Granada nutmegs are not inferior but are liable to be confused with the Papuan (New Guinea) false long nutmeg derived from *M. argentea*, which is of inferior quality, but resembles the genuine in external appearance.—*Bull. imp. Inst., Lond., 1933, 31, 2, 197.*

NICOTINA

The specification of the Association of British Insecticide Manufacturers (Bulletin No. 82: Ministry of Agriculture and Fisheries) for nicotine requires the material to be free from coal tar bases and the nicotine content to be declared.

A tentative method for the determination of free nicotine in tobacco is described in *Methods of Analysis (A.O.A.C., 1940, 141)*. The powdered sample is stirred with water and the pH of the latter then measured in a quinhydrone or hydrogen electrode. The nicotine content is found from a curve plotted from data given for pH and nicotine content. An official method for the determination of nicotine in tobacco insecticides is also described (*ibid.*, 64). The sample is steam-distilled with aqueous alkali and paraffin and the distillate collected in hydrochloric acid. When the liquid distilling is free from nicotine (as shown with silico-tungstic acid) the distillate is adjusted to volume and filtered. An aliquot is treated with dilute hydrochloric acid and silico-tungstic acid (12% of $4H_2O \cdot SiO_2 \cdot 12NO_3 \cdot 22H_2O$) and stood overnight. The collected precipitate is washed with hydrochloric acid and ignited. Wt. of residue $\times 0.1140$ = nicotine.

The determination of nicotine by the process of Phyl and Schmidt depends upon the precipitation of the distillate (neutralised to methyl red) with 0.05M picric acid, and titration of the washed picrate in water and toluene mixture with N/10 sodium hydroxide using phenolphthalein indicator. The volume of distillate is reduced by saturating the liquor with salt, using magnesia for liberation of the nicotine.

Determination in Forensic Material. Mix the material, finely chopped, with citrate buffer and papayotin, the proteolytic enzyme from papaya, previously activated with hydrocyanic acid. Preserve with toluene and incubate at 40° for 48 hours with frequent shaking. Heat for 1 hour on a water-bath, filter,

wash residue with acidulated water, and evaporate the combined filtrates to small volume. Extract with ether, acidified, once, and with ether in strongly alkaline solution three times. Treat the ethereal extracts with a solution of picric acid in ether, filter off the precipitated nicotine dipicrate and weigh.—F. Halstrom, per *Chem. Abstr.* 1939, 89.

Insecticides. The Association of British Insecticide Manufacturers have prepared approved specifications with method of analysis, for certain insecticides and fungicides. By requiring such substances to comply with the specifications, purchasers are able to obtain standard products of high quality.

The products include lead arsenate in powder or paste, lime-sulphur solution, nicotine and nicotine sulphate, copper sulphate, burgundy powder, cheshunt compound, soft soaps for spraying, sodium, potassium and calcium cyanides, and formaldehyde.—Bulletin No. 82, 1934, Ministry of Agriculture and Fisheries.

Anabasine—Distinction from Nicotine. Anabasine, $C_{10}H_{14}N_2$, an insecticidal alkaloid from *Anabasis aphylla* (Chenopodiaceæ) occurs commercially as the sulphate in the form of a syrupy liquid almost identical in appearance and odour with commercial nicotine sulphate. It may be distinguished by adding to an ethereal solution of iodine. Nicotine gives a turbidity which, in a closed test-tube, settles into large red crystals of the periodide. Anabasine solutions remain clear.—S. A. Katz, *Z. anal. Chem.*, 1937, 108, 408.

NITROGLYCERINUM

Liquor Glycerylis Trinitratis (B.P.). Determined by digesting in a closed tube with one-tenth its volume of sodium hydroxide solution for 1 hour, transferring with alcohol to a brine-charged nitrometer and shaking with equal volumes of potassium iodide solution and dilute sulphuric acid; each millilitre of nitric oxide produced at 15.5° and normal pressure is equivalent to 0.00505 g. of $C_3H_5O_9N_3$; from 0.9 to 1.1% *w/v* should be indicated. Alcohol content, 88 to 90% *v/v* of ethyl alcohol. Spiritus Glycerylis Trinitratis, *U.S.P. XII*, contains from 1 to 1.1% of $C_3H_5(NO_2)_3$, determined by the general method for nitrites after absorption in sodium hydroxide solution, as described under Spiritus Æthylis Nitritus, *U.S.P. XII*, the iodine liberated on addition of potassium iodide to an acidified solution being titrated with N/10 sodium thiosulphate. Nitroglycerinum solutum, *P.G. VI*, and Nitroglycerinum solutum, *P. Helv. V*, contain 0.98 to 1.02% of $C_3H_5(ONO_2)_3$; assayed similarly by addition of N/2 alcoholic potash, water and hydrogen peroxide solution, warming till decolorised, and titrating the warm solution with N/2 hydrochloric acid to phenolphthalein; a blank titration is performed. 1 ml. N/2 potash = 0.0022706 g. nitroglycerin.

An official quantitative method for the determination of glyceryl trinitrate (nitroglycerin) is described in *Methods of Analysis* (A.O.A.C., 1940, 478).

Determination in Tablets. One tablet reputed to contain 1/100 gr. is crushed finely and macerated in 2.5 ml. of glacial acetic acid for 2 hours. 1 ml. of the filtered liquid is transferred to a suitable 10 ml. tube and, with the tube immersed in cold water, 0.25 ml. of a 0.5% solution of diphenylamine in nitrogen-free sulphuric acid is added. The tube is agitated in the cold water and after 3 minutes diluted to the 10 ml. mark with glacial acetic acid. The depth of colour is then compared with that given by similarly and simultaneously treating 2.4 ml. of standard solutions of KNO_3 in glacial acetic acid. For 1/100 gr., 1/150 gr. and 1/200 gr. trinitrin, standard solutions of KNO_3 should contain

per 100 ml. of glacial acetic acid 0.0340 g., 0.0231 g. and 0.0173 g. respectively.—C. H. Sykes, *Pharm. J.*, ii/1933, 267.

In tablets or solution, nitroglycerin can be determined by reduction with Devarda alloy and distillation of the ammonia in standard sulphuric acid. A quantity of the preparation representing about 0.05 g. of nitroglycerin is mixed in an 800 ml. Kjeldahl flask with 50 ml. of a saturated sodium sulphate solution, 150 ml. of water and sufficient 10% sulphuric acid to make the mixture acid to litmus. The mixture is distilled into a flask containing 30 ml. of 5% sodium hydroxide solution and reduction effected by means of 2 g. of Devarda alloy.—E. L. Anderson, *J. Ass. off. agric. Chem., Wash.*, 1932, 140.

The U.S.P. XII gives the following method for Tabellæ Glycerylis Trinitratis, containing 80 to 112% of the labelled amount of $C_3H_5(NO_3)_3$. Triturate in a mortar tablets equivalent to 0.05 g. of glyceryl trinitrate with 50 ml. of ether. Decant the ether into a stoppered flask and extract a further 4 times with 30 ml. portions of ether; shake the flask for 15 minutes and filter into a beaker, washing with 50 ml. of ether. Cautiously evaporate the ether to about 5 ml. and transfer with ether to an 800 ml. Kjeldahl flask containing 100 ml. of water and add a mixture of 30 ml. of N/1 sodium hydroxide, 60 ml. of water and 10 ml. of potassium permanganate solution (5%), followed by 100 ml. of water; mix, stand for 30 minutes, and then heat on a water-bath for 30 minutes. After cooling add 3 g. of Devarda's alloy, stopper the flask immediately with a stopper carrying a scrubber, and connect with a vertical condenser, the receiver containing 50 ml. of N/50 sulphuric acid. Heat gently while hydrogen is evolved and then distil two-thirds of the liquid. Back titrate with N/50 sodium hydroxide using methyl red indicator and run a blank determination. 1 ml. N/50 sulphuric acid = 0.001514 g. $C_3H_5(NO_3)_3$.

IMPROVED METHODS FOR TABLETS. (a) Place five tablets in a 500 ml. Kjeldahl flask, add 25 ml. of saturated sodium sulphate solution, 75 ml. of water and sufficient sulphuric acid to make just acid to litmus paper (usually 0.3 ml. of N/1 sulphuric acid is required). Distil just to dryness, using a still head, into a flask containing 10 ml. of N/10 sodium hydroxide, keeping the outlet tube below the surface of the alkali. Wash down the condenser and outlet tube and evaporate the sodium hydroxide solution to dryness. Add 2 ml. of water, 0.3 g. (± 0.01 g.) of reduced iron and 2 ml. of 50% v/v sulphuric acid, allow to stand for 10 minutes and boil for 2 minutes. Transfer the acid solution to a steam distillation apparatus, make alkaline with 4 ml. of saturated sodium hydroxide solution, and distil the liberated ammonia into a flask containing 10 ml. of N/10 sulphuric acid until the distillate measures 500 ml. Take 100 ml. of the distillate, add 2 ml. of Nessler's reagent and compare the colour produced with that produced by adding the same amounts of reagent to 100 ml. of a solution containing ammonium chloride equivalent to 0.1 mg. of nitrogen. The colours produced are most easily compared in a colorimeter, but a tintometer or even Nessler glasses could be used for the purpose. Whatever means of comparing the colours is used the colour of the unknown should not vary more than 20% from that of the standard. A control experiment must always be carried out exactly as described.—Wilfred Smith, *Quart. J. Pharm.*, 1935, 370.

(b) Add the powdered tablets equivalent to about 1 ml. of nitroglycerin to 5 ml. of glacial acetic acid in a stoppered cylinder and shake for 1 hour. Filter and to 1 ml. add 2 ml. of phenoldisulphonic acid, stir and stand for fifteen minutes. Dilute with 8 ml. of water, make alkaline with ammonia, cool, adjust to 20 ml. at 20° and filter. Compare the colour with controls prepared with a known solution of nitroglycerin or with 1 ml. of a 0.225% silver nitrate solution. The colour produced in each case should be equal to 7.0 Lovibond yellow units when viewed in a cell 1 inch internal width.—H. O. Meek, *Quart. J. Pharm.*, 1935, 375.

The second report of the Commission Internationale des Spécialités includes an agreed method for the determination of glyceryl trinitrate in tablets in which the medicament is extracted with chloroform and an aliquot part of the chloroform solution diluted with ether is mixed with alcohol and alkali and allowed to stand for 10 minutes. After evaporation on a water-bath the residue is transferred to a 100 ml. flask using about 90 ml. of water, 2 ml. of Griess-Romijn reagent added, water added to volume and the colour produced in 15 to 30 minutes is compared with that obtained by similarly treating a standard sodium nitrite solution.

Griess-Romijn Reagent. α -Naphthylamine 1 g., sulphanilic acid 10 g., tartaric acid 89 g., in water 900 g. .

Erythritylis Tetranitras Dilutus (B.P.). Contains from 47·5 to 52·5% of $C_4H_6O_{12}N_4$. Estimated by the Kjeldahl method, using salicylic acid with the sulphuric acid for digestion; a blank estimation is performed using half the weight of lactose; each millilitre of N/10 sulphuric acid is equivalent to 0·007552 g. of $C_4H_6O_{12}N_4$. Erythritylis Tetranitras Dilutus, *U.S.P. XI*, is replaced by Tabellæ Erythritylis Tetranitratis, *U.S.P. XII*. Assayed by repeated extraction with ether, evaporation below 35° and drying over sulphuric acid for 18 hours, the tablets are required to contain 93 to 107% of the amount stated on the label.

NUX VOMICA

Nux Vomica (B.P.). Contains not less than 1·2% of strychnine and not more than 1% of foreign organic matter. Assayed by continuous extraction with a mixture of alcohol, dilute solution of ammonia and chloroform (12:1:4), followed by evaporation of the solvent and extraction with sulphuric acid from a chloroform mixture; the total alkaloids are then extracted with chloroform from ammoniacal solution, the solvent evaporated, alcohol added, and evaporated; the residue is dissolved in 15 ml. of 3% sulphuric acid and 2 ml. of nitric acid, allowed to stand for 30 minutes at 15° to 20°, made alkaline with sodium hydroxide and extracted with chloroform; the residual strychnine, after drying for 30 minutes at 100°, is titrated with N/10 sulphuric acid to methyl red or cochineal, multiplying the result by 1·02 to correct for loss.

Nux Vomica, *U.S.P. XII*, contains not less than 1·15% of strychnine; assayed by the aliquot part method, maceration with ether-chloroform and extraction of the total alkaloids with chloroform and ammonia from the acid solution; the total alkaloid is purified by transferring again to acid and a further extraction with chloroform and ammonia; after evaporation of the chloroform the alkaloidal residue is dissolved in 15 ml. of sulphuric acid (3%) and 1·5 ml. of nitric acid mixed with 1·5 ml. of sodium nitrite (5%) solution is added; after 10 minutes only, the strychnine is extracted from the liquid made alkaline with sodium hydroxide (10%) solution with chloroform, finally titrating the strychnine; no correction for loss of strychnine is used.

Noix Vomique, *Fr. Cx.* 1937, determined by titration after extraction with ether-chloroform, contains 2 to 3% of total alkaloids of which not less than 45% is strychnine; for strychnine separation the first titration liquid with sulphuric and nitric acids is kept at 50° to 60° for 10 minutes, extracted with chloroform and ammonia, transferred to acid, finally extracted with ether-chloroform, dried at 105° and the residue of strychnine weighed. Semen Strychni, *P.G. VI*, contains not less than 5% of total alkaloid of

Strychnos nux vomica Linné. Semen Strychni, *P. Helv. V*, contains not less than 2.5% of total alkaloids and the powder for administration is adjusted with lactose to contain 2.5% of total alkaloids. *I.A.* (Second) recommended a standard of 2.5% of total alkaloids.

Strychnos cinnamomifolia from Travancore. Total alkaloidal content from 2.432 to 2.801%, only about 0.3% being strychnine. The seeds closely resemble those of *S. Nux-vomica*, but have not been commercially exploited.—G. R. A. Short, *Yearb. Pharm.*, 1924, 646.

Benzol (*B.P.* '98) is not such a good solvent for strychnine as chloroform but is advised, in preference to the latter, if used in larger quantity for extraction in the estimation process: it does not emulsify.—H. Deane, *Pharm. J.*, ii/1924, 96. See also D. B. Dott, *ibid.*, 251.

Assay. The following method is claimed to give more consistent results than those usually employed. In the case of the powdered seed 4.5 g. of the material is first shaken with 15 g. of chloroform and 30 g. of ether; for the extract, 0.75 g. is dissolved in 10 ml. of water with the addition of 1 ml. of dilute sulphuric acid, and shaken with chloroform and ether; for the tincture, 45 g. is treated with 1 ml. of dilute sulphuric acid, evaporated to about 5 g. and treated with 15 g. of chloroform and 30 g. of ether. In all cases 6 g. of 25% sodium carbonate solution is added, and the alkaloids are shaken into the chloroform-ether mixture. The mixture is cleared if necessary by the additions of powdered tragacanth, and strained through cotton wool, 30 g. of the filtrate being then evaporated nearly to dryness and treated with 10 ml. of N/10 hydrochloric acid and 20 ml. of water. The remainder of the chloroform is evaporated off and, after cooling, the mixture is filtered through cotton wool, the filter being washed with water to make the total volume up to about 100 ml. This solution is now titrated with N/10 sodium hydroxide, using methyl red as indicator. One ml. of the acid is taken as equivalent to 0.0364 g. of strychnine+brucine.—A. Jermstad, *Norsk. farm. Tidsskr.*, 1936, 44, 375, 388.

A colorimetric method for the determination of strychnine in nux vomica and its preparations is described. The procedure involves the rapid extraction of the total alkaloids by addition of piperazine to the powdered drug followed by treatment with boiling tetrachloroethylene, transference of the extracted alkaloids to dilute aqueous sulphuric acid and isolation of the strychnine as the ferrocyanide. The determination is completed by applying Malaquin's colour test as modified by Deniges.—J. Allen and N. Allport, *Quart. J. Pharm.*, 1940, 252.

The potency of nux vomica preparations as indicated by their toxicity is not in agreement with the results obtained by chemical assay for total alkaloids. Although better agreement is sometimes obtained between toxicity and strychnine content, in other samples marked discrepancies were observed.—J. C. Munch *et al.*, *J. Amer. pharm. Ass.*, 1937, 29.

Nux Vomica Pulverata (B.P.). Adjusted with nux vomica of higher or lower alkaloidal content, or with lactose, to contain 1.14 to 1.26% of strychnine. Ash, not more than 3%.

Extractum Nucis Vomicae Siccum (B.P.). After extraction with alcohol (50%) and completion of the assay as for Nux Vomica, it contains 4.75 to 5.25% of strychnine; Extractum Nucis Vomicae, *N.F. VII*, contains 7 to 7.75% of strychnine. Assayed by digestion with dilute alcohol acidified with acetic acid and after addition of chloroform and ammonia, proceeding as for Fluidextract of Nux Vomica, *N.F. VII*.

Extractum Nucis Vomicae Liquidum (B.P.). Contains 1.425 to 1.575% w/v of strychnine.

Tinctura Nucis Vomicae (B.P.) contains 0.119 to 0.131% *w/v* of strychnine. The tincture of the *U.S.P. XII* contains 0.105 to 0.125% *w/v* of strychnine.

Brucina Sulphas (N.F. VII). $(C_{23}H_{18}O_4N_2)_2 \cdot H_2SO_4 \cdot 7H_2O = 1013.1$. Loss at 100°, not more than 13%; ash, not more than 0.1%. A test for strychnine is included in which after destroying the brucine with nitric and sulphuric acids, making alkaline, extracting with chloroform and evaporating, the residue should produce no violet colour with sulphuric acid and potassium dichromate.

Strychnina (B.P.C.). $C_{21}H_{22}O_2N_2 = 334.2$. By titration in excess N/10 sulphuric acid with N/10 sodium hydroxide to cochineal, a purity of not less than 99% should be indicated. Ash, not more than 0.1%. A limit test for brucine is included; no reddish colour should be produced with a mixture of equal parts of nitric acid and water. *Strychnina, N.F. VII*, should yield not more than 0.1% of ash.

Extraction from Viscera. In the extraction of alkaloidal poisons from viscera the laborious and time-consuming Stas-Otto process may be replaced by treatment of the minced material with trichloroacetic acid. This at once yields a water-clear filtrate free from protein and fat and containing the whole of the alkaloid originally present. From this filtrate the alkaloid can conveniently be removed by absorption on kaolin, from which after neutralisation it is eluted by hot chloroform.—C. P. Stewart, S. K. Chatterji and S. Smith, *Brit. med. J.*, ii/1937, 790.

Spectrum of Strychnine. The smallest quantity, e.g., 1/500 grain, can be detected—useful in cases of poisoning. Alkaloids generally give characteristic spectra.—Prof. J. J. Dobbie.

The strychnine compound of bordeaux B (strychnine azorubrate) can be decomposed during the assay of preparations containing strychnine and bordeaux B by allowing sodium hydroxide to act on the substance for two or three days before extracting with an organic solvent. This gives an almost theoretical yield of pure strychnine.—J. B. Dott, *Pharm. J.*, ii/1939, 527.

Strychninae Hydrochloridum (B.P.). $C_{21}H_{22}O_2N_2 \cdot HCl \cdot 2H_2O = 406.7$. Should lose not less than 7% and not more than 9% at 110°. Ash, not more than 0.1%.

Liquor Strychninae Hydrochloridi (B.P.). Contains 0.95 to 1.05% *w/v* of strychnine hydrochloride. Determined by extraction with chloroform from ammoniacal mixture, drying at 100° and titration with excess N/10 sulphuric acid and N/10 sodium hydroxide, using methyl red or cochineal indicators.

Volumetric estimation of Liq. *Strychninae*, using N/10 potassium dichromate, which precipitates the alkaloid quantitatively from slightly acid solution.—J. Rae, *Pharm. J.*, i/1928, 270.

Strychninae Nitras (B.P.C.). $C_{21}H_{22}O_2N_2 \cdot HNO_3 = 397.2$. Ash, not more than 0.1%. Complies with limit tests for brucine and sulphate. The *N.F. VII* salt should yield not more than 0.1% of ash and comply with tests for acidity, brucine, chloride and sulphate.

Strychninum nitricum is the only form of strychnine official in the *P. Helv. V*. When titrated with N/10 sodium hydroxide using phenolphthalein as indicator, it should contain not less than 99.5% of $C_{21}H_{22}O_2N_2 \cdot HNO_3$.

Tabellæ Strychninæ Nitratis (N.F. VII). Tablets of 20 mg. or more contain 92.5 to 107.5%, tablets of 20 mg. to 1.2 mg. contain 91 to 109% and tablets of less than 1.2 mg. contain 88 to 112%, of the labelled amount of strychnine nitrate, including all tolerances. Assayed by macerating with water and dilute sulphuric acid overnight, filtering and extracting with chloroform an aliquot of the filtrate, made alkaline with ammonia solution, removing the chloroform and dissolving the residue in N/20 sulphuric acid, finally back titrating with N/50 sodium hydroxide using methyl red indicator.

Strychninæ Phosphas (N.F. VII). $C_{21}H_{22}O_2N_2 \cdot H_3PO_4 \cdot 2H_2O = 468.4$. By extraction of the alkaloid with chloroform from a solution rendered ammoniacal and final titration with N/10 sulphuric acid, using methyl red indicator, it yields 70 to 73% of strychnine. Complies with tests for readily carbonisable substances, chloride, sulphate and brucine.

Strychninæ Sulphas (B.P.C.). $(C_{21}H_{22}O_2N_2)_2 \cdot H_2SO_4 \cdot 5H_2O = 856.5$. Loss at 100° , 9 to 11%. Ash limit, 0.1%. Complies with the limit test for brucine. Strychninæ Sulfas, *U.S.P. XII*, leaves not more than 0.1% of ash, loses not more than 11.5% at 105° and complies with tests for acidity and brucine; 0.2 g. complies with the specified test for carbonisable substances.

Tabellæ Strychninæ Sulfatis (U.S.P. XII). Contain 93 to 107% of the labelled amount of $(C_{21}H_{22}O_2N_2)_2 \cdot H_2SO_4 \cdot 5H_2O$ for tablets of 20 mg. or more, and 90 to 110% for tablets of less than 20 mg., including all tolerances. Assayed by macerating overnight a weighed quantity of powdered tablets with water and dilute sulphuric acid, filtering and extracting the alkaloid with chloroform from an aliquot of the filtrate made alkaline with ammonia solution. To the combined extracts 10 ml. of dehydrated alcohol is added for each 50 ml. of chloroform and the mixture evaporated almost to dryness. The residue is dissolved in alcohol and N/50 sulphuric acid and the excess acid titrated with N/50 sodium hydroxide using methyl red indicator.

Garratt (*Drugs and Galenicals*) recommends determination of small amounts of strychnine by the method of Scandola (*Analyst*, 1911, 144). The residue containing about 0.1 mg. is treated with 0.1 g. ammonium vanadate and 3 drops of sulphuric acid, diluted to volume, and matched against a control solution.

Determination of Strychnine in Presence of Caffeine. For determining small quantities of strychnine in the presence of caffeine, e.g., in Syr. Glycero-phosph. Co., the following procedure is suggested. To 50 ml. of syrup add 5 g. of citric acid, make alkaline with ammonia and extract five times with chloroform. Evaporate the solvent and weigh the anhydrous residue. Dissolve in 25 ml. of water containing 1 ml. of 25% *v/v* sulphuric acid, add 1 ml. of freshly prepared 5% potassium ferrocyanide, stir, and allow to stand overnight. Filter, wash well with slightly acidified water, place the funnel in the neck of a separator, pierce the filter paper and wash in the precipitate successively with 10 ml. of 10% ammonia, 10 ml. of water and 10 ml. of chloroform. Extract three times with chloroform, evaporate with 5 ml. of alcohol, dry, and weigh.—D. C. Garratt, *Analyst*, 1937, 538.

Determination of Small Quantities. Strychnine in amounts as low as 5 mg. can be determined by precipitation with a standard potassium mercuric iodide solution and back-titration of the excess iodide with potassium iodate. The reagent is prepared by dissolving 0.46 g. of mercuric iodide and 0.40 g. of potassium iodide in water to 100 ml. and is standardised against M/100 potassium iodate solution (1.0701 g. dried at 120° in 1% hydrochloric acid to 500 ml.). The strychnine is dissolved in 2 to 3 ml. of dilute acid, a known volume of reagent added (at least 1 ml. for each 3 mg. of strychnine), the mixture diluted to 25 ml. in a graduated flask and allowed to stand in the dark for 1 to 2 hours. It is then filtered, rejecting the first 2 to 3 ml. of filtrate, and 20 ml. is transferred to a stoppered bottle. 16 ml. of concentrated hydrochloric acid is added, the mixture cooled and then titrated with iodate solution, chloroform being added to indicate the end-point. 1 ml. of M/100 iodate solution is equivalent to 2.228 mg. of anhydrous alkaloid, 2.648 mg. of anhydrous nitrate and 2.855 mg. of sulphate ($5H_2O$). The process is applicable to tablets containing sucrose, lactose or starch.—A. G. Murray, *J. Ass. off. agric. Chem., Wash.*, 1937, 638.

Official methods for the determination of strychnine in tablets and liquid preparations (other alkaloids being absent) are described in *Methods of Analysis* (A.O.A.C., 1940, 591).

Denigès' modification of Malaquin's colour test for strychnine has been applied to the determination of strychnine in hypodermic tablets and injections. Tables are given correlating the value of the red component of the colour obtained to the alkaloidal content.—N. L. Allport and N. R. Jones, *Quart. J. Pharm.*, 1942, 245.

Picrotoxinum (U.S.P. XII). M.p., 198° to 200°. Complies with tests for absence of alkaloids.

ŒSTRADIOL

(with other ŒSTROGENS, PROGESTERONE and the ANDROGENS)

Œstradiol (B.P.C. Supp. IV). $C_{18}H_{24}O_2 = 272.2$. M.p., 175° to 178°. α_D of 1% solution in dioxan, +75° to +82°. α_D at 17°, of the *Fr. Cx.* substance, determined on a 1% solution in dioxan, +80°.

Œstradiolis Benzoas (B.P.C. Supp. IV). $C_{25}H_{28}O_3 = 376.2$. M.p., 189° to 195°. α_D of 1% w/v solution in dioxan, +58° to +61°. Loss at 100°, not more than 0.5%. Ash, not more than 0.1%. Preparations may be assayed by comparison with the standard by a method depending on its power to promote in adult female rats or mice, completely deprived of their ovaries, the cellular changes in the vaginal secretion characteristic of normal Œstrus. The potency may be stated in units, the unit being the Œstrus-producing activity of 0.0001 mg. of the standard preparation of pure crystalline Œstradiol monobenzoate issued by the National Institute of Medical Research, London, and adopted as the International Standard. *Œstradiolis Benzoas*, U.S.P. XII, melts between 191° and 196°; α_D determined on a 2% solution in dioxan, +58° to +63°. α_D of the *Fr. Cx.* substance, determined on a 1% solution in dioxan, +57.5°.

Œstriol (B.P.C. Supp. IV). $C_{18}H_{24}O_3 = 288.2$. M.p., about 282°. α_D of 1% solution in dioxan, +53° to +63°.

Œstronum (B.P.C. Supp. IV). $C_{18}H_{22}O_2 = 270.2$. M.p., 255° to 260°. α_D of 1% w/v solution in dioxan, +157° to +167°. Loss at 100°, not more than 0.5%. Ash, not more than 0.1%. May be assayed by biological comparison with the standard preparation of pure crystalline Œstrone issued by the National Institute for Medical Research, London. *Œstronum*, U.S.P. XII, melts between 258° and 261°; α_D at 25°, determined on a 1% solution in dioxan, +155° to +165°. The substance of the *Fr. Cx.* melts at 259° to 260°, α_D at 15° of a 1% solution in dioxan, +165°.

Colorimetric Assay. The following method is based on the coupling of oestrone with *p*-diazobenzenesulphonic acid to give a red azo dyestuff which is compared with that of a standard produced by coupling β -naphthol in the same way. The reaction depends on the presence of a phenolic group and is therefore given by both oestrone and oestriol.

The reagent is prepared by diazotising sulphanilic acid. 4.5 g. of the acid is dissolved in 500 ml. of water containing 45 ml. of concentrated hydrochloric acid. To 1.5 ml. of this solution is added 1.5 ml. of sodium nitrite solution (5% NaNO_2). The mixture is immersed in an ice-bath and after 5 minutes 6 ml. more of the nitrite solution is added. The solution is again cooled for 5 minutes in the ice-bath and then diluted to 50 ml. with water. The diluted acid is kept in the ice-bath and used not earlier than 15 minutes nor later than 24 hours after its preparation.

To (1-x) ml. of water is added 5 ml. of 1.1% anhydrous sodium carbonate solution and 2 ml. of the diazobenzenesulphonic acid reagent. Exactly 30 seconds later is added x ml. of the oestrone solution and 0.5 ml. of 10% NaOH . 1 ml. of dilute β -naphthol solution containing 0.01 mg. per 100 ml. is treated similarly and the colours compared in a colorimeter.

Assuming that equimolecular amounts of oestrone and β -naphthol take part in the reactions it was found that 0.01875 mg. of oestrone corresponded to 0.01 mg. β -naphthol. Using a solution of oestrone standardised in rat units it was found that 1 mg. = 2666 r.u.—M. J. Schmulovitz and H. B. Wylie, *J. biol. Chem.*, 1936, 116, 415.

Oestroni Benzoas (*B.P.C. Supp. IV*). $\text{C}_{25}\text{H}_{26}\text{O}_3 = 374.2$. M.p., about 220° . α_D of 1% solution in dioxan, about $+120^\circ$. M.p. of the substance of the *Fr. Cx.*, 218.5° to 221° ; α_D at 15° of 1% solution in dioxan, $+128^\circ$.

Stilboestrol (*B.P. Add. VI*). $\text{C}_{18}\text{H}_{20}\text{O}_2 = 268.2$. M.p., 168° to 171° . Loss at 100° , not more than 0.5%. Produces a clear or only slightly turbid solution with 30 parts of water and 30 parts of N/1 sodium hydroxide. Ash, not more than 0.1%. Assayed by acetylation and drying the acetate at 100° , it contains not less than 99% of $\text{C}_{18}\text{H}_{20}\text{O}_2$.

Colour Tests. *A. Violet Colour Reaction.* Mix equal volumes of 1% solution of stilboestrol and 5% v/v solution of bromine, both in glacial acetic acid, and warm in a water-bath for 1 minute. One drop of the resulting orange solution mixed with 5 drops of absolute alcohol followed by 2 ml. of water produces a deep violet colour. *B. Green Colour Reaction.* A green colour is produced when the 1% solution of stilboestrol is heated with the bromine solution in the proportion of 5 atoms of bromine per molecule of stilboestrol. In the presence of sucrose, e.g., sugar-coated tablets, the colour produced is a deep blue changing to dull purple and orange red.

The reactions are not given by stilboestrol dipropionate, stilboestrol acetate or hexaestrol. Dienaestrol gives both reactions but requires only about half the amount of bromine to produce a green colour. Details are given for the application of the first test to the rough colorimetric determination of stilboestrol.—T. T. Cocking, *Analyst*, i/1943, 144.

Stilboestrolis Dipropionas. $\text{C}_{32}\text{H}_{40}\text{COOC}_2\text{H}_5 \cdot \text{C}_2\text{H}_5 \cdot \text{C}(\text{C}_2\text{H}_5)_2 \cdot \text{C}(\text{C}_2\text{H}_5)_2 \cdot \text{C}_6\text{H}_4 \cdot \text{COO} \cdot \text{C}_2\text{H}_5$. M.p., about 103° . Saponification value, about 290. Ultra-violet absorption in dehydrated alcohol at $240 \text{ m}\mu$ (calculated on the substance dried at 100°), 390.

Biological Assay. Stilboestrol was tested against oestrone to find how much of each substance was required to produce oestrus in 50% of a large group of castrated rats. A curve could be drawn by plotting dosage against percentage in oestrus. Using the figure of 50% to indicate 1 unit, this was found to be 3 microgrammes as a single injection in oil for oestrone and 0.95 microgrammes for stilboestrol, while for multiple aqueous injections, the unit was 0.95 microgrammes for oestrone and 0.62 microgrammes for stilboestrol.—V. L. Koenig and R. G. Gustavson, *J. Pharmacol.*, 1940, 69, 355.

Progesteronum (*B.P.C. Supp. III*). $C_{21}H_{30}O_2 = 314.2$. M.p., 128° to 131° . α_D of 1% solution in dehydrated alcohol, $+183^\circ$ to $+194^\circ$. Loss at 100° , not more than 0.5%. Ash, not more than 0.1%. Hormone du Corps Jaune, *Fr. Cx.* 1937, should give a dioxime melting at 246° to 248° ; m.p., 121° ; α_D (0.775% solution in chloroform), $+200^\circ$; possesses the same activity as the international standard.

The international unit is defined as the activity of 1 mg. of the chemically pure substance, the activity being measured by the pregravid changes occurring in the uterine horn of immature female rabbits.

Assay. The induction of sexual receptivity in the ovariectomised guinea-pig previously conditioned by theelin serves as a sensitive and specific method for the assay of progesterone. As little as 0.05 i.u. can be detected and only one-tenth of the amount of progesterone needed in other methods of assay is required.—R. Hertz, R. K. Meyer and M. A. Spielman, *Endocrinology*, 1937, 533.

Testosteronum (*B.P.C. Supp. III*). $C_{19}H_{28}O_2 = 288.2$. M.p., 151° to 156° . α_D of 1% solution in dioxan, $+104^\circ$ to $+112^\circ$. Loss at 100° , not more than 0.5%. Ash, not more than 0.1%.

Detection of Ketones of the Sterol Group. Steroid ketones having a double bond conjugated with the keto group, such as testosterone, progesterone, corticosterone, etc., usually possess greater biological activity than the corresponding saturated ketone. A polarographic method of detection is described that is claimed to have many advantages over the existing colorimetric and spectrophotometric methods. Details are also given for the estimation of testosterone in an alcoholic testicle extract.—J. Eisenbrand and H. Picher, *Z. physiol. Chem.*, 1939, 260, 83.

Testosteroni Propionas (*B.P.C. Supp. III*). $C_{22}H_{32}O_3 = 344.25$. M.p., 118° to 122° . α_D of 1% solution in dioxan, $+83^\circ$ to $+90^\circ$. Loss at 100° , not more than 0.5%. Ash, not more than 0.1%.

OLEA ESSENTIALIA

The essential oils and natural perfumes are obtained by distillation, solvent extraction and cold and hot enfleurage processes. **Distillation** yields a true volatile oil but is not suitable where the yield of oil is very small or the product is damaged by heat. The principles of the distillation of essential oils are described in "A Treatise on Distillation," by Durrans (*Perfum. essent. Oil Rec.*, 1920, 154). **Solvent extraction** has largely displaced the enfleurage process for many perfumes, benzene and petroleum ether being used as solvents. The product obtained by the evaporation of the solvent contains, besides the odorous bodies, resins, waxes and colouring matter; these are separated more or less completely by treating the extract with alcohol, refrigerating, filtering and evaporating the alcohol to obtain "*absolutes*." Many perfumes are made by this process, of which the more usual are jasmine, orange-flower, carnation, violet, lilac, rose, cassia, and oak moss.

The enfleurage process, in which the odour of the flowers is taken up by repeatedly spreading fresh flowers on trays coated with fat, or by immersing them in melted fat, is still used for jonquil, jasmine, tuberose and a few others. The fat when sufficiently saturated, is extracted with alcohol, which is evaporated to leave the "absolute." Some account of the process is given in *Chem. & Drugg.*, i/1927, 319.

For further information as to sources, constituents, methods of distillation, yields, analytical and physical data, etc., see *Die Ätherischen Öle*, by Gildermeister and Hoffman; *Chemistry of Essential Oils and Artificial Perfumes*, by E. J. Parry; *Essential Oils*, by H. Finckmore; also Umney, *Perfum. essent. Oil Rec.*, July, 1912; and Bovill, *Mfg Chem.*, Jan.-Feb., 1932. For the chemistry of the terpenes, see Durran, *Perfum. essent. Oil Rec.*, 1929, 278, also L. Ruzicka, *ibid.*, 1934, 85 and 117.

Determination of Aldehydes. The following method is recommended by the Essential Oil Sub-Committee of the Committee on Uniformity of Analytical Methods for the determination of aldehydes in oils of cassia, cinnamon, lemon-grass, orange, bitter almond, cherry-laurel, cummin and terpeness oils of lemon and orange:—Weigh out exactly, into a glass-stoppered tube approximately 150 mm. long and 25 mm. in diameter, a suitable quantity of the oil, add 5 ml. of benzene and 15 ml. of N/2 hydroxylamine hydrochloride reagent; shake vigorously and titrate with N/2 alcoholic potash (60% alcohol) until the red colour changes to yellow; continue the shaking and titrating until the full yellow colour of the indicator is permanent in the lower layer after shaking vigorously for 2 minutes and then allowing to stand for the liquids to separate. The report should be consulted for further details and for the different factors employed.—*Analyst*, 1934, 105.

Determination of Esters. A method has been recommended by the Essential Oil Sub-committee of the Analytical Methods Committee. The report states that unless the conditions of the test are strictly adhered to considerable variations in results are likely to occur between different operators and different laboratories; the published details should therefore be studied. 2 ml. of oil (or other suitable quantity so that the amount of alkali added is at least double that required for saponification) is weighed into the saponification flask, 5 ml. of freshly well-boiled neutralised alcohol added and the free acid titrated with N/10 alcoholic potassium hydroxide using 0.2 ml. of 0.2% solution of phenolphthalein in alcohol (60%) as indicator. To the neutralised solution is added 20 ml. of N/2 alcoholic potassium hydroxide, and the mixture is boiled for 1 hour, at the end of which it is immediately titrated with N/2 acid using an additional 0.5 ml. of phenolphthalein solution. A blank determination is carried out simultaneously. The difference between the two titrations is calculated to the percentage of esters as usual, using the appropriate factor. Those for the most commonly occurring esters are:—

| | | | |
|--------------------|--------|----------------------|--------|
| Bornyl acetate .. | 0.0981 | Santalyl acetate .. | 0.1311 |
| Geranyl acetate .. | 0.0981 | Methyl salicylate .. | 0.0760 |
| Linalyl acetate .. | 0.0981 | Geranyl tiglate .. | 0.1181 |
| Menthyl acetate .. | 0.0981 | Linalyl benzoate .. | 0.1290 |

With oil of sweet birch and oil of wintergreen the free acid should be determined in a separate experiment by shaking 5 g. with 25 ml. of water and titrating with N/10 aqueous sodium hydroxide using phenol red as indicator. The saponification is carried out on the unneutralised oil and an allowance made for the free acid in calculating the results. The boiling to effect saponification should be continued for 1½ hours.—Report of Essential Oil Sub-Committee to the Analytical Methods Committee, per *Analyst*, 1937, 541.

Micro-Chemistry in the Domain of Essential Oils and Perfumery Materials. Apparatus and methods are described, qualitative tests, and characteristics of materials.—Rosenthaler, *Perfum. essent. Oil Rec.*, 1930, 277.

Oils are identified by the microscopical examination of the crystalline compounds of their constituents with potassium hydroxide, phenylhydrazine and sodium phenate, in various solvents.—W. Green, *Perfum. essent. Oil Rec.*, 1939, 309.

Determination of Alcohols in Essential Oils. A method using orthophosphoric acid as a catalyst. Acetylation is completed in about fifteen minutes at ordinary temperatures. The process is not satisfactory for oil of citronella.—Sabatay, *C. R. Acad. Sci., Paris*, 1934, 199, 1419.

Identification of Phenols. Methods given for the identification of thymol, carvacrol, eugenol, iso-eugenol, and vanillin through the melting-points of the arylacetic acids.—Reed, *Perfum. essent. Oil Rec.*, 1933, 190.

The Determination of Solubilities. Report of Sub-Committee on Uniformity of Analytical Methods. The terms used to describe solubility are described.—*Analyst*, 1930, 386. The alcohols used in the solubility tests for volatile oils should comply with the sp. gr. limits—90%, 0.8334 to 0.8340; 80%, 0.8634 to 0.8640; 70%, 0.8896 to 0.8901.

Solubilities of perfumery ingredients, essential oils and synthetics in ethyl alcohol.—*Perfum. essent. Oil Rec.*, 1924, 283.

Essential oils are able to hold water in solution, particularly those which contain a large proportion of oxygenated bodies.—see Umney and Bunken, *Perfum. essent. Oil Rec.*, May, 1912.

Determination of Essential Oils in Solution in Alcohol. A volume of the sample containing about 200 mg. of oil (or 8 g. of sample if it contains less than 1% of oil) is weighed by difference into a 100 ml. separating funnel and shaken with 80 ml. of 30% ammonium sulphate solution. When the oil has separated the aqueous layer is poured through a small filter over which 1 g. of medicinal Norit carbon has been distributed, the separating funnel and filter being washed with two successive quantities, each of 5 ml., of the ammonium sulphate solution. The filtrate can be used for the determination of the alcohol after acidification with sulphuric acid. The filter and contents are then shaken in the separating funnel with 15 ml. of ether and sufficient anhydrous sodium sulphate to absorb the water and ethereal extract is poured through a small filter into a tared 150 ml. flask containing a weighed amount (about 0.5 g.) of liquid paraffin. The separating funnel and filter are washed five times using 5 ml. of ether each time, and the solvent is finally removed by distillation at not over 40°. The last traces are removed by a current of air and the residue is placed in a vacuum desiccator over calcium oxide until the difference in weight between two successive weighings does not exceed 1 to 2 mg.—H. J. Van Giffen, *Pharm. Weekbl.*, 1936, 641.

Rapid method of determination. The oil is extracted from the solution by means of petroleum ether, which is then evaporated in a special apparatus and the residue weighed; the alcohol is determined by distilling the extracted liquid and taking the specific gravity of the distillate.—G. A. Rosenberger, *Perfum. essent. Oil Rec.*, 1939, 133.

Determination of Volatile Oil in Drugs. The methods for the determination of essential oils in drugs involve the use of a continuous distillation apparatus in which the drug is distilled in steam and the condensed water, separated from the oil, is returned to the distilling flask, the amount of oil obtained being measured at the end of the operation. Considerable variation has often been found in the yields of oil from different samples of a drug, and it is desirable that minimum limits for the yield of the essential oil in such drugs should be fixed. A method for certain drugs is given in the *U.S.P. XII* and in *P.G. VI*. Apparatus and processes have also been described by J. F. Clevenger (*Perfum. essent. Oil Rec.*, 1928, 226), G. R. A. Short (*Quart. J. Pharm.*, 1931, 444), Cocking and Middleton (*ibid.*, 1932, 521, also 1935, 435), Kuhn (*Pharm. Ztg.*, 1934, 99, per *Quart. J. Pharm.*, 1934, 691), Horkeimer (*Quart. J. Pharm.*, 1935, 535), Kofler and Herrens-schwand (*ibid.*, 1936, 117), Wasecky, Graf and Bayer (*ibid.*, 1936, 308), Mijnhardt (*ibid.*, 1936, 572), Rom (*ibid.*, 1936, 701), H. J. Van Giffen (*ibid.*, 1938, 269), K. Koch (*ibid.*, 1939, 275),

L. Goldberg, R. K. Sugden, E. H. Wirth and F. N. Gathercoal (*J. Amer. pharm. Ass.*, 1938, 385).

The volatile oil contents of drugs and spices may be determined with apparatus in which the receiver is a standard separating funnel receiver (V. 4) as specified by the Standardisation of Tar-Products Tests Committee. Results are given for a number of substances including nutmeg, cinnamon, clove, caraway, cardamom and umbelliferous fruits.—C. E. Sage and H. R. Fleck, *Analyst*, 1934, 614.

The various methods which have been advocated are compared and details of design and use of an apparatus are described. Sources of error are discussed, including the loss of oil through grinding the material and loss of yield through the degree of comminution being insufficient to allow the oil to escape from the tissues. Figures are given comparing the powdered and unground drugs of commerce for a very large number of samples, also a table showing the yields obtained when the drugs were distilled whole and in varying degrees of comminution.—H. O. Meek and F. G. Salvin, *Quart. J. Pharm.*, 1937, 471.

Surface Tension of essential oils in dynes per centimetre at 20°.—*Perfum. essent. Oil Rec.*, 1935, 18.

Changes in Refractive Index with alteration in temperature. A table giving correction per 1°.—*Perfum. essent. Oil Rec.*, 1937, 95.

Detection of Traces of Metallic Contamination. The organic matter is first destroyed by oxidation with a mixture of sulphuric and nitric acids and a solution obtained. An aliquot part of this solution is used for the determination of the metal present. Methods are given for lead, copper, tin, iron, aluminium and zinc.—F. R. Nares, *Quart. J. Pharm.*, 1935, 255.

Absorption of Ultra-violet Light by Essential Oils. Absorption curves using ultra-violet light have been determined for a large number of essential oils. The curves can be used for identification, detection of adulteration and in certain cases for quantitative assay.—D. Van Os and K. Dykstra, *J. Pharm. Chim. Paris*, 1937, 25, 437, 485; per *Quart. J. Pharm.*, 1938, 122.

Chloramine Value. The number of mls. of N/100 chloramine solution used up by 0.05 g. of substance. It is found by dissolving less than 50 mg. of oil in glacial acetic acid, and adding an equal volume of N/5 chloramine solution in a four-fold excess of water. Stand one hour in the dark, add potassium iodide solution and titrate with sodium thiosulphate. The chloramine value often decreases with age. Values for a number of oils are given.—P. W. Danchwortt and J. Hotzel, per *Quart. J. Pharm.*, 1938, 285.

Diene Value. The diene value of an essential oil is the number of grammes of iodine equivalent to the maleic anhydride absorbed by 100 g. of the substance. The method involves the heating of a weighed amount of the substance with a measured volume of a standardised maleic anhydride solution in a suitable solvent in sealed tubes for a definite period followed by a volumetric determination of the maleic anhydride remaining unchanged. The diene value may prove of use in the detection of diluents foreign to particular oils.—T. F. West, *Perfum. essent. Oil Rec.*, 1940, 20.

TERPENELESS ESSENTIAL OILS

Essential oils from which the terpenes and sesquiterpenes have been separated by fractional distillation *in vacuo* have the advantage that they are stronger in perfume and flavour and more readily soluble in diluted alcohol than the original oils. Some oils, e.g., clove oil, contain so small a proportion of terpenes that there is generally no point in rendering them terpeneless. In other oils the proportion of the terpenes is so variable that the yield of terpeneless oil differs widely. With the exception of the citrus oils, which are prepared in both the terpeneless and sesquiterpeneless forms, the term terpeneless generally implies an oil which has been obtained by fractionation and is free from both terpenes and sesquiterpenes.

In a new method for the production of terpeneless oils advantage is taken of the selective solubility of two solvents, pentane for the terpenes and diluted methyl alcohol for the oxygenated bodies. The process is covered by patent.—van Dijck and Ruys, *Perfum. essent. Oil Rec.*, 1937, 91.

The following table is mostly derived from Durrans, *Perfum. essent. Oil Rec.*, 1924, 240, with revisions, 1929. In the original article a number of physical data are also given.

Terpeneless and Sesquiterpeneless Oils

| | Concentration | 1 vol. Oil soluble in Alcohol |
|--|---------------|--------------------------------|
| Absinthe | — | 2 to 3 vols. 70% |
| Angelica | 20 | 3 vols. 70% |
| Aniseed | 1.5 | 10 vols. 80% |
| Bay | 2—3 | 1—1.5 vols. 70% |
| Bergamot | 2.5—3 | 1 vol. 80%; 3—4 vols. 70% |
| Calamus | — | 25 vols. 60%; 3 vols. 70% |
| Cananga | 6 | 1 vol. 90% |
| Caraway | 2 | 2 vols. 70%; 19 vols. 50% |
| Cardamom | 2 | 2—3 vols. 70% |
| Cassia | 2 | 2 vols. 70% |
| Cedarwood | — | 1 vol. 90% |
| Celery | 8 | 2 vols. 80% |
| Cinnamon Leaf | 3 | 1 vol. 70%; 3 vols. 60% |
| Citronella (Ceylon) | 2 | 2 vols. 70% |
| Citronella (Java) | 1.5 | 2 vols. 70% |
| Clove | 1.5 | 2.5 vols. 60%; 1 vol. 70% |
| Coriander | 1.5 | 2 vols. 70% |
| Cumin | 1.5—2 | 5—7 vols. 70% |
| Dill | 2—3 | 2—3 vols. 70% |
| Eucalyptus Globulus | 2—3 | 2 vols. 70% |
| Fennel | 1.5 | 1 vol. 90% |
| Geranium | 1.5—2 | 1—2 vols. 70% |
| Ginger | 12 | 2—4 vols. 80% |
| Grape Fruit | 50 | 2 vols. 80% |
| Hops | 8 | 1 vol. 80%; 20—30 vols. 70% |
| Juniper Berry | 10 | 1 vol. 90% |
| Lavender (French) | 2 | 1—2 vols. 70% |
| Lemon | 25 | 3 vols. 70% |
| Lemongrass | 1.5 | 2 vols. 70% |
| Linaloe | 1.5 | 1.5—3 vols. 70% |
| Limes (Handpressed) | 6 | 2 vols. 70% |
| Limes (Distilled) | 15—20 | 1.5 vols. 70% |
| Mandarin Orange | 70 | 2.5—4 vols. 70%; 1.5 vols. 80% |
| Neroli | 2 | 2—2.5 vols. 70% |
| Nutmeg | 8 | 1 vol. 80%; 4 vols. 70% |
| Orange | 65 | 2—3 vols. 70% |
| Palmarosa | 1.5 | 2 vols. 70% |
| Parsley | 10 | 2 vols. 80% |
| Patchouli | 4 | 1 vol. 95% |
| Pennyroyal | 2 | 2 vols. 70% |
| Peppermint (American) | 2 | 3 vols. 70% |
| Peppermint (Jap. dementolised) | 2 | 2.5 vols. 70%; 6 vols. 60% |
| Peppermint (Mitcham) | 2 | 2.5—3 vols. 70% |
| Petitgrain | 2 | 1 vol. 80%; 3 vols. 70% |
| Pimento | 1.5 | 1 vol. 70% |
| Pinus Sibirica (Abies) | 2—3 | 3 vols. 70% |
| Rosemary (French) | 2 | 2—3 vols. 75% |
| Rose Otto (Stearopteneless) | 1.5—2 | 1—1.5 vols. 70% |
| Sage | 6 | 2—2.5 vols. 75% |
| Sandalwood | 1.5 | 3—5 vols. 70% |
| Sassafras | 2 | 1 vol. 90% |
| Spearmint | 4 | 2 vols. 75% |
| Spike Lavender | 2 | 2 vols. 70% |
| Spike (Spanish) | 1.5 | 2 vols. 70% |
| Thyme | 2—3 | 2—3 vols. 70% |
| Vetivert | 10 | 1 vol. 80% |
| Ylang (Manilla) | 4—5 | 1—1.5 vols. 90% |

ANTISEPTIC POWERS OF ESSENTIAL OILS

The antiseptic power of a number of essential oils was determined by W. H. Martindale, *Perfum. essent. Oil Rec.*, Nov., 1910. The *Lancet* carbolic acid coefficient using *B. Coli communis* was determined either in aqueous or in saponaceous solutions according to the solubility of the particular oil.

| Essential Oil | Carbolic acid co-efficient | Chief chemical constituents |
|---------------------------------|----------------------------|--|
| Origanum Oil (A) | 26 | 82% phenols, e.g., carvacrol. |
| Thymol (S) | 25 | |
| Carvacrol (S) | 21 | |
| Thymol (A) | 19 | |
| Thyme Oil (S) | 15 | 46% phenols (thymol, etc.). |
| Thyme Oil (A) | 13 | 46% phenols (thymol, etc.). |
| Geraniol (S) | 12 | |
| Cinnamon Leaf Oil (S) .. | 10 | 86% phenols, e.g., eugenol. |
| Cinnamon Bark Oil (S) .. | 9 | 52% aldehyde, e.g., cinnamic. |
| Clove Oil (S) | 9 | 90% phenols, e.g., eugenol. |
| Cinnamic Aldehyde (S) .. | 8 | |
| Citronellol (S) | 8 | |
| Cinnamon Bark Oil (S) .. | 8 | 82% aldehyde, e.g., cinnamic. |
| Cinnamon Bark Oil (A) .. | 7 | 82% aldehyde, e.g., cinnamic. |
| Rosemary Oil (S) | 6 | |
| Otto of Rose (S) | 6 | 68% alcohols estimated as geraniol. |
| Cassia Oil (S) | 5 | 83.5% aldehyde, e.g., cinnamic. |
| Wintergreen Oil (S) | 5 | Methyl salicylate. |
| Eucalyptus Amygd. (S) .. | 5 | Phellandrene and eucalyptol. |
| Lavender Oil (English) (S) .. | 5 | Esters as linalyl acetate, 11%. Other constituents of the oil are linalool as such, esters other than the acetate, cineole and limonene. |
| Lemon Oil (S) | 4 | Limonene, citral, 4 to 7% citronellal, geranyl acetate, possibly other esters of geraniol and citronellal. |
| Almond Oil, Ess. s.A.P. (S) .. | 4 | Benzaldehyde chiefly. |
| Eucalyptol (S) | 4 | |
| Eucalyptus Glob. Oil (S) .. | 4 | 67% eucalyptol together with pinene, phellandrene, alcohols and aldehydes. |
| Garlic Oil | 2 | Allyl sulphide chiefly. |
| Light Oil of Tar (Rect.) (S) .. | 2 | Volatile bodies. Contains no phenols. |
| Santal Oil (S) | 1½ | 93.8% alcohol calculated as santalol, $C_{16}H_{34}O$. |
| Birch Tar Oil (S) | 1½ | Stated to contain guaiacol, cresol and pyrocatechin. |
| Cade Oil (S) | 1 | |

(A) = Aqueous solution. (S) = Saponaceous solution.

These values have been confirmed and others added to the list by later investigators. The following are some of the more important references:—

Carbolic acid coefficients are given against a mixed culture of *Micrococcus catarrhalis* for a number of compounded perfumes. Isopropyl alcohol solutions have slightly greater bactericidal powers than ethyl alcohol solutions.—Dyche-Teague, *Perfum. essent. Oil Rec.*, 1924, 6, 40 and 181; see also Bryant, *ibid.*, 252.

Germicidal value of some Australian essential oils. Certain oils could be used in place of phenol or cresols as preservatives in antisera.—Penfold, *Perfum. essent. Oil Rec.*, 1924, 127.

Rideal-Walker Coefficients of Constituents of Essential Oils

The following list has been compiled from tables appended to an article on "An investigation into the germicidal powers and capillary activities of certain essential oils," by S. Rideal, E. K. Rideal and A. Sciver (*Perfum. essent. Oil Rec.*, 1928, 285).

| Substance | R.W. coeff. | Type | Remarks |
|--------------------------------|-------------|-----------------|--------------------------------|
| Acetophenone.. | 4 | Ketone .. | Synthetic. |
| Amyl salicylate .. | 4 | Ester .. | — |
| Amyl valerianate .. | 5 | Ester .. | — |
| Anethole .. | 11 | Ether .. | — |
| Anisaldehyde .. | 7 | Aldehyde .. | — |
| Anthranilic acid .. | 12 | Acid .. | In alcohol. |
| Aromadendrene .. | < 1 | Sesquiterpene.. | <i>E. nova-anglica.</i> |
| Australol .. | 22.5 | Phenol.. | <i>E. cneorifolia.</i> |
| Benzaldehyde.. | 9 | Aldehyde .. | Synthetic. |
| Benzyl acetate .. | 2 | Ester .. | — |
| Benzyl alcohol .. | 5.25 | Alcohol .. | — |
| Benzyl valerianate .. | 6 | Ester .. | — |
| Borneol .. | 15 | Alcohol .. | In alcohol. |
| Borneol .. | 10 | Alcohol .. | In soap solution. |
| Bornyl acetate .. | 6 | Ester .. | Synthetic. |
| Butyl valerianate .. | 10 | Ester .. | — |
| Camphor .. | 6 | Ketone .. | <i>Doryphora sassafras.</i> |
| Cineol .. | 3.5 | Oxide .. | <i>E. polybractea.</i> |
| Cinnamic aldehyde .. | 17 | Aldehyde .. | Synthetic. |
| Citral .. | 19.5 | Aldehyde .. | <i>E. Staigeriana.</i> |
| Citronellal .. | 13.5 | Aldehyde .. | <i>E. citriodora.</i> |
| Citronellol .. | 14 | Alcohol .. | Redn. of citronellal. |
| Citronellyl valerianate .. | 2 | Ester .. | Synthetic. |
| Coumarin .. | 4 | Lactone .. | — |
| Croweacin .. | 2 | Ether .. | <i>Eriostemon Croweii.</i> |
| Cryptal .. | 12 | Aldehyde .. | <i>E. cneorifolia.</i> |
| Cuminal .. | 12.75 | Aldehyde .. | <i>E. cneorifolia.</i> |
| Cymene .. | 8 | Hydrocarbon .. | Synthetic. |
| Darwinol .. | 13 | Alcohol .. | <i>Darwinia grandiflora.</i> |
| Darwinol acetate .. | 3 | Ester .. | <i>Darwinia grandiflora.</i> |
| Ethyl valerianate .. | 4.5 | Ester .. | Synthetic. |
| Eudesmol .. | < 1 | Sesquiterpene.. | <i>E. Moorei.</i> |
| Eugenol .. | 15 | Phenol .. | <i>Doryphora sassafras.</i> |
| Geraniol .. | 21 | Alcohol .. | Geranyl acetate. |
| Geranyl acetate .. | 0 | Ester .. | <i>E. Macarthuri.</i> |
| Geranyl valerianate .. | 2 | Ester .. | Synthetic. |
| Isoamyl alcohol .. | 2 | Alcohol .. | Synthetic. |
| Isobutyl alcohol .. | 2 | Alcohol .. | — |
| Isobutyl valerianate .. | 8.5 | Ester .. | — |
| Isomenthone .. | 14 | Ketone .. | Redn. of piperitone. |
| Isosafrol .. | 12 | Ether .. | Synthetic. |
| Isovaleraldehyde .. | 5 | Aldehyde .. | <i>E. Macarthuri.</i> |
| Limonene .. | 1 | Terpene .. | <i>E. Staigeriana.</i> |
| Linalyl acetate .. | 5.25 | Ester .. | Synthetic. |
| Linalool .. | 13 | Alcohol .. | <i>Geijera</i> species. |
| Menthol .. | 20 | Alcohol .. | All sources. |
| Menthyl .. | 19 | Alcohol .. | Old stock. |
| Menthone .. | 10 | Ketone .. | Redn. of piperitone. |
| Menthyl valerianate .. | 3 | Ester .. | Synthetic. |
| Methyl anthranilate .. | 6.5 | Ester .. | — |
| Methyl eugenol ether .. | 13.5 | Ether .. | — |
| Methyl salicylate .. | 5.5 | Ester .. | — |
| Ocimene .. | 3 | Olefine .. | <i>Homoranthus flavescens.</i> |
| Phellandral .. | 9.25 | Aldehyde .. | <i>E. cneorifolia.</i> |
| Phenyl ethyl valerianate .. | 4 | Ester .. | Synthetic. |
| <i>l</i> - α -Pinene .. | 1 | Terpene .. | <i>E. phlebophylla.</i> |

Rideal-Walker Coefficients of Constituents of Essential Oils (contd.)

| Substance | R.W. coeff. | Type | Remarks |
|-------------------------|-------------|-------------|-----------------------------|
| Piperitol | 13 | Alcohol .. | <i>E. radiata.</i> |
| Piperitone | 8 | Ketone .. | <i>E. dives.</i> |
| Piperitone | 8 | Ketone .. | Commercial 80%. |
| Piperonal | 3 | Aldehyde .. | Synthetic. |
| Propyl valerianate .. | 8 | Ester .. | — |
| Rhodinyl valerianate .. | 1 | Ester .. | — |
| Safrol | 11 | Ether .. | <i>Doryphora sassafras.</i> |
| Terpin hydrate | 1 | — .. | Pinene. |
| Terpineol | 7.5 | Alcohol .. | <i>E. australiana.</i> |
| α -Thujone | 12 | Ketone .. | <i>Boronia thujona.</i> |
| Thymol | 25 | Phenol .. | Ajowan or piperitone. |
| Valerianic acid | 2 | Acid .. | Synthetic. |
| Vanillin | 3.5 | Aldehyde .. | — |
| Xylene musk | 4 | — .. | — |

Rideal-Walker Coefficients of Essential Oils

| Oil | Rideal-Walker coefficient |
|-----------------------------|---------------------------|
| Bay | 5.5 |
| Bois de Rose | 5.4 |
| Cajuput | 1.0 |
| Cassia | 1.4 |
| Cedarwood | 1.6 |
| Ceylon Citronella | 2.0 |
| Cinnamon Leaf | 7.5 |
| Cloves | 8.0 |
| Coriander | 5.4 |
| Eucalyptus | 1.6 |
| Japanese Mint (Dementh.) .. | 0.7 |
| Java Citronella | 2.2 |
| Juniper Berry | Less than 0.1 |
| Kananga | Less than 0.1 |
| Lavender | 1.6 |
| Lemon | 0.4 |
| Orange | 0.4 |
| Palmarosa | 9.0 |
| Patchouli | 1.6 |
| Sassafras | 0.6 |
| Spearmint | 2.8 |
| Spike Lavender | 1.6 |
| Star Aniseed | 0.4 |
| Sweet Birch | 0.4 |
| White Camphor | 0.4 |
| Wormseed | 1.0 |

—S. Rideal, E. K. Rideal and A. Sciver, *Perfum. essent. Oil Rec.*, 1928, 285.

The vapours of some essential oils have disinfecting activity with selectivity towards acid-fast bacteria.—Otto Schöbl, *Philipp. J. Sci.*, 1924, 443, per *Perfum. essent. Oil Rec.*, 1924, 330.

Oils of high carboic acid coefficient are more likely to lower surface tension.—E. K. Rideal, S. Rideal and A. Sciver, *Perfum. essent. Oil Rec.* 1928, 235.

Physiological aspects of essential oils in relation to constitution. A table is given of Rideal-Walker coefficients for a large number of constituents of essential oils.—Malcolm Dyson, *Perfum. essent. Oil Rec.*, 1930, 287.

Germicidal values of commercial eucalyptus oils. The cineole-containing oils have not the highest antiseptic value.—Penfold, *Bull. tech. Mus., Sydney*, 1933, No. 2; also *Perfum. essent. Oil Rec.*, 1935, 130.

Ti-tree oil has been introduced and recommended for its bactericidal properties. Numerous articles have appeared on its composition and use, see *Perfum. essent. Oil Rec.*, 1929, 155; *Mfg Chem.*, 1936, 332; *Chem. & Drugg.*, 1936, 524; *Pharm. J.*, 1936, 632. See also p. 268.

Antiseptic Power of Essential Oils. The addition of certain fixative substances increased the time during which oils are effective.—Risler, *Quart. J., Pharm.*, 1937, 152.

The use of *Bact. typhosum* as the sole test organism in the Rideal-Walker test is shown to be unreliable for comparing the relative efficiencies of germicidal preparations containing essential oils such as pine-oil or titree oil, or combinations of these oils with chlorinated phenols, particularly where the preparations are to be used at considerable dilution for the purposes of skin disinfection or application to wounds. It is doubtful if a more suitable test organism than *Staph. aureus* can be obtained since, besides being one of the normal skin flora, it is the most resistant of the non-spore-forming pathogens.—Q. Moore and J. N. Walker, *Pharm. J.*, ii/1939, 507.

A study of the penetration power of antiseptics using a modification of the Reddish cup method for volatile substances shows that the inorganic acids and bases in general exhibit high penetration, the organic acids, picric, salicylic, benzoic and others, show slight penetration values. Organic preparations have variable penetration values; acriflavine, gentian violet and safranin show small penetration. The ointments in general show slight penetration values. Essential oils exhibit a wide variation of values; oils of bitter almonds and mustard show the highest values; oils of cinnamon, turpentine and thyme and eucalyptol show medium values; oils of cloves, eucalyptus, lavender, lemon, caraway, pimento and rosemary give values somewhat higher than 5% PhOH which has a penetration value of 5 mm.—L. J. Piccoli and Morris Hecht, *J. Amer. pharm. Ass.*, 1939, 157-62.

Report on twenty-two essential oil-bearing materials and seventeen pure oils. The pH and bacterial count examined over a period of eight days. Infusions of fennel, marjoram, juniper berry, galangal and orris root possess some bactericidal power.—H. Kliewe and E. K. Huthmacher, *Perfum. essent. Oil Rec.*, 1939, 61.

NOTES ON INDIVIDUAL ESSENTIAL OILS

For notes on essential oils not included in the following group, see under the corresponding drug, e.g., notes on *Oleum Caryophylli* are included under *Caryophyllum*.

OLEUM ABIETIS

Oleum Abietis (*B.P. Add. I*). Contains 33 to 45% w/w of esters, calculated as bornyl acetate, $C_{19}H_{30}O_2$. Sp. gr., 0.905 to 0.925. α_D , -32° to -45° . n_{D20} , 1.466 to 1.476. Soluble in an equal volume of alcohol (90%). Assayed by the *B.P.* method for esters, by saponification of 2 g. of the neutralised oil with 40 ml. of N/2 alcoholic potash during one hour on a water-bath.

The following limits are recommended for the Tyrolean oil of *Abies alba*:—sp. gr. at 15° , 0.867 to 0.880; optical rotation, -13° to -67° ; refractive index at 20° , 1.4729 to 1.4760; ester content (calculated as bornyl acetate) up to 11%; solubility at 20° , 1 in 4 to 10 volumes of alcohol 90%, sometimes with slight turbidity. Since as much as 55% and more distils below 170° , any definite

figure would be meaningless.—C. H. Grimm, E. E. Langenau and E. S. Guenther, *J. Amer. pharm. Ass., Sci. Edn.*, 1941, 209.

In addition to oil of Siberian fir, there are several **pine-needle oils** of commercial importance. Oil of *Pinus sylvestris* has very variable characters according to the locality in which it is produced. Oil of *Pinus pumilio* was official in the B.P. '98; it is mainly produced in the Tyrol.

An account of the process for the production of resin and volatile oils. A fraction of the volatile oil is sold as steam-distilled pine oil which contains a high proportion of alcohols, mostly α -terpineol.—*Chemical Age*, 1938, 39, 387.

Yields of pine-needle oils from various species of Abietineæ.—Willner, *Quart. J. Pharm.*, 1933, 253.

ABIETIC ACID derivatives and decomposition products.—*J. chem. Soc. Abstr.*, i/1920, 232.

OLEUM AURANTII

Oleum Aurantii (B.P.C.). Residue on evaporation 2 to 4% w/w; sp. gr., 0.848 to 0.852 (sweet orange) or 0.852 to 0.856 (bitter orange). α_D , +95° to +99° (sweet orange) or +88° to +96° (bitter orange). n_{D20} , 1.472 to 1.474 (sweet orange) or 1.472 to 1.475 (bitter orange). The first 10% of the distilled oil has an optical rotation the same or only slightly lower than the original oil. Oleum Aurantii, U.S.P. XII, is derived from fresh peel of the sweet orange only; sp. gr., 0.842 to 0.846 at 25°; α_D , +94° to +99° at 25°; n_{D20} , 1.4723 to 1.4737. Residue on evaporation not above 100°, not less than 1.7%. The first 10% of the distilled oil has an optical rotation the same or not more than 2° away from that of the original oil and a refractive index from 0.0008 to 0.0015 lower at 20°. Oleum Aurantii Amari, N.F. VII, has a sp. gr. at 25° of 0.845 to 0.851; α_D at 25°, +88° to +98°; the first 12% of the distilled oil has an optical rotation not more than 2° lower or more than 4° higher than the original oil.

The difference between bitter and sweet orange oils is not greater than the variations of analytical characters, flavour and odour so that they cannot be distinguished. **Tangerine orange** oil differs greatly from orange oil in flavour and aroma; it contains methyl anthranilate and methyl methylantranilate, and yields a distinctive terpeneless oil.

The principal varieties of orange oil commercially are Sicilian, West Indian, Californian, and recently S. African. The last two show a low residue on evaporation.

South African orange oil. Yield of oil and physical data.—*Perfum. essent. Oil Rec.*, 1930, 111.

Composition of Californian orange and lemon oils. A very complete report with methods of analysis and tabulated results.—Poore, U.S. Dept. of Agriculture, Tech. Bull. No. 241, per *Perfum. essent. Oil Rec.*, 1932, 166.

Residue on evaporation and other data.—C. T. Bennett, *Perfum. essent. Oil Rec.*, 1932, 2.

The appearance under ultraviolet light of the oils of mandarin, sweet orange, bergamot and lemon; undiluted and in alcoholic solution of 0.17% and 0.0034% showed marked differences particularly in the undiluted oils. Spots on porcelain of the undiluted oils showing mandarin, purple; sweet orange, mustard yellow; bergamot, greenish yellow, purple halo; lemon, deep greenish yellow, violet halo. An abstract of the original paper of E. Bottini in *Annali della Sperimentazione Agraria*, appears in *Perfum. essent. Oil Rec.*, 1935, 85.

Terpeneless Oil of Sweet Orange. Berte (*Yearb. Pharm.*, 1924, 102) gives yield of terpeneless oil as 1.5% and the following figures:—Sp. gr., 0.883 to 0.900; α_D , +25° 50' to +42° 20'; aldehydes as citral, 25% to 43%; esters as linalyl acetate, 14.8%. The sp. gr. is sometimes higher, up to 0.915.

A note from Sicily says the process of manufacture is exactly similar to that for terpeness lemon oil, q.v., except that a larger quantity of terpenes are distilled off—about 95%. No physical or chemical data are known for the finished product, as it is only very rarely distilled, and then it is not a great success. The odour of the terpeness orange oil does not pay for the distillation in many cases. The terpeness orange oils on the market are usually "synthetic" products, i.e., a mixture of which the chief odoriferous constituent is methyl methylantranilate. The distillation in London and elsewhere is carried out more scientifically than in Southern Italy.

OLEUM BERGAMOTTÆ

Oleum Bergamottæ (B.P.C.). Contains not less than 36% of esters, calculated as linalyl acetate, $C_{12}H_{20}O_2$. Sp. gr., 0.882 to 0.886; α_D , +12° to +24°; n_{D20° , 1.464 to 1.467. Residue on evaporation, 4 to 5% w/w, and the residue has an acid value of 20 to 50, and saponification value of 160 to 200. Complies with tests for absence of certain artificial esters, terpinyl acetate, and glyceryl acetate. *Oleum Bergamottæ, N.F. VII*, contains not less than 36% of ester, as linalyl acetate; sp. gr., 0.875 to 0.880 at 25°; α_D at 25°, +8° to +24°. Tests for fixed oils and for chlorinated compounds are included.

Determination of adulteration with terpinyl acetate.—Schimmel's Reports, 1911, 116, per *Yearb. Pharm.*, 1912, 75. Advantage is taken of the fact that terpinyl acetate is much less readily saponified than linalyl acetate.

Appearance under ultra-violet light.—See notes under *Oleum Aurantii*.

OLEUM CAJUPUTI

Oleum Cajuputi (B.P.). Contains 50 to 60% w/w of cineole, $C_{10}H_{18}O$. Sp. gr., 0.916 to 0.926. α_D , not greater than -4°. n_{D20° , 1.462 to 1.472. Soluble in 2 volumes of alcohol (80%). Assayed by determination of the freezing-point of a mixture of 3 g. of the oil and 2.1 g. of *o*-cresol, the freezing-point being between 27.4° and 35.1°. The *B.P. Add. I* alters the standard for cineole content and the refractive index—cineole content, 50 to 65% w/w; n_{D20° , 1.464 to 1.472. *Oleum Cajuputi, U.S.P. X*, should be soluble in 1 volume of 80% alcohol; sp. gr., 0.912 to 0.925 at 25°; n_{D20° , 1.4660 to 1.4710. *Oleum Cajuputi, P. Helv. V*, contains 45 to 70% of cineole; assayed by crystallising, by cooling in ice water for 30 minutes, 2.5 g. with 2.5 g. eucalyptol and 10 ml. of 50% resorcinol solution, separating the crystals, decomposing by warming with dilute sodium hydroxide solution and measuring the volume of cineole.

This is now required to be rectified, a process which increases the proportion of cineole. The upper limit of 60% for cineole is too low and excludes many high quality oils. The limit should be raised to 65%.—C. T. Bennett, T. T. Cocking and W. H. Simmons, *Pharm. J.*, ii/1933, 581.

OLEUM CEDRI

Oleum Cedri (B.P.C.). Sp. gr., 0.941 to 0.950; α_D , -25° to -46°; n_{D20° , 1.495 to 1.510. *Oleum Cedri Folii, U.S.P. XII*,

assayed by interaction with hydroxylamine hydrochloride, contains not less than 60% thujone, $C_{10}H_{16}O$. Sp. gr., 0.910 to 0.920 at 25°; α_D at 25°, -10° to -13° ; n_{D20° , 1.4560 to 1.4590.

EAST AFRICAN CEDARWOOD OIL, from *Juniperus procera*, has an odour similar to that of American cedarwood oil, but the optical rotation is higher.—*Bull. Imp. inst. Lond.*, 1931, 29, 430; per *Quart. J. Pharm.*, 1932, 101.

MICROSCOPICAL CEDARWOOD OIL for use with immersion objectives and condensers, is a specially prepared oil, more viscous than the ordinary, and with refractive index adjusted to a definite figure. The oil to be used as a clearing agent in microscopy is the ordinary variety.

OLEUM CHENOPODII

Oleum Chenopodii (B.P. Add. I). Contains not less than 65% w/w of ascaridole, $C_{10}H_{16}O_2$. Sp. gr., 0.962 to 0.983; α_D , -4° to -8° ; n_{D20° , 1.474 to 1.479. Assayed by titration of the iodine liberated when an acetic acid (90%) dilution of the oil is allowed to interact for 5 minutes with a potassium iodide solution, hydrochloric acid and glacial acetic acid at -3° ; a blank titration is conducted, diluting with water before titration (see T. T. Cocking and Hymas, *Quart. J. Pharm.*, 1930, 253). The U.S.P. XII assays by a similar method and requires the same ascaridole content. Sp. gr., 0.950 to 0.980 at 25°; n_{D20° , 1.4740 to 1.4790. Essence de Chénopode Vermifuge, *Fr. Cx.* 1937, contains not less than 60% ascaridole.

A statistical study of the physical constants of 39 samples of the oil. Ascaridole is in direct relationship to the sp. gr. and in inverse relationship to the solubility in alcohol (70%).—J. C. Munch and W. F. Reindollar, *J. Amer. pharm. Ass.*, 1931, 564.

Determination of Ascaridole. The following method depends upon the liberation of iodine by ascaridole from a strongly acidified solution of potassium iodide. The reaction is complex and conditions must be carefully controlled. About 2.5 g. of the oil is weighed in a 50 ml. graduated flask which is then filled to the mark with 90% acetic acid, and some of this solution is placed in a narrow bore burette graduated in 20ths of a ml. and capable of being read to 0.01 ml. The stopcock should be such that it is possible to run out 5 ml. in not more than 5 seconds. Into a stoppered tube (150 mm. long and 25 mm. diameter) place 3 ml. of 5N potassium iodide solution, 10 ml. of glacial acetic acid and 5 ml. of hydrochloric acid. Stopper the tube and cool to -3° in a freezing mixture, then run in from the burette, as rapidly as possible, 5 ml. of the acetic acid solution of the oil; replace the stopper, mix the contents and allow to stand in a cool place for 5 minutes during which the temperature of the contents will rise but must not exceed 10° . After allowing about 2 minutes for the walls of the burette to drain completely note the volume run out. After the tube has been allowed to stand for 5 minutes titrate directly with N/10 sodium thiosulphate without the addition of starch. Conduct a blank determination in the same way but diluting the mixture with 20 ml. of water before titrating with thiosulphate, and deduct the volume used from the amount used in the titration. Each ml. of N/10 sodium thiosulphate is equivalent to 0.00665 g. of ascaridole.—Report of the Essential Oils Sub-Committee to the Analytical Methods Committee, *Analyst*, 1936, 179.

A tentative method for the determination of ascaridole in oil of chenopodium is described in *Methods of Analysis (A.O.A.C., 1940, 625)*. 10 ml. of a 1% dilution in 95% alcohol and 50 ml. of a titanous chloride solution are boiled for 2 minutes in a flask fitted with a bunsen valve and back titrated with standard ferric ammonium sulphate solution using ammonium thiocyanate indicator (1 g. ascaridole is reduced by 1.284 g. of $TiCl_3$).

A comparative study of the three recognised assays for oil of chenopodium. The methods of the *A.O.A.C.*, the *B.P.* and the *U.S.P.* are compared. The first two determine ascaridole, while the last measures the acetic-acid soluble fraction. The author finds that the two former methods yield results concordant with each other, but at variance with those obtained by the *U.S.P.* method.—W. F. Reindollar, *J. Amer. pharm. Ass.*, 1939, 589.

OLEUM CITRONELLÆ

Oleum Citronellæ (*B.P.C.*). Estimated by the *B.P.* method for alcohols, which consists of determination of the ester values of the acetylated oil and of the original oil and calculation from a formula, it contains not less than 57% (for the Ceylon oil) or not less than 85% (for the Java oil) by weight of total acetylisable constituents calculated as geraniol. Sp. gr., 0.897 to 0.912 (Ceylon) or 0.885 to 0.900 (Java); α_D , -6° to -14° (Ceylon) or -2° to -5° (Java); n_{D20} , 1.479 to 1.485 (Ceylon) or 1.468 to 1.473 (Java). Yields a clear or slightly opalescent solution with 10 parts of alcohol (80%) and no globules separate after 24 hours at a temperature not lower than 15.55° .

Examination of Citronella Oil. A method is given for the estimation of geraniol in the presence of citronellal. The oil is oxidized by the method of Dupont and Labaune, the N determined by the Kjeldahl method giving the citronellal; another portion of the oxidized oil is acetylated and the geraniol content found. Oils from Java, Celebes and Sumatra were examined.—Reclaire and Spoelstra, *Perfum. essent. Oil Rec.*, 1927, 130.

Determination of citronellal by means of hydroxylamine hydrochloride used at a low temperature.—*Analyst*, 1932, 773.

Formylation method shown to be unreliable.—C. T. Bennett, *Perfum. essent. Oil Rec.*, 1921, 351.

Alcohol as an adulterant.—E. J. Parry, *Chem. & Drugg.*, ii/1923, 390.

Determination of aldehydes. For a method of avoiding the difficulty caused by the insolubility of the citronellal sulphonate, as occurs in the usual bisulphite method, see F. D. Dodge, *Amer. Perfum.*, 1929, 24, 11; per *Quart. J. Pharm.*, 1929, 328.

OLEUM EUCALYPTI

Oleum Eucalypti (*B.P.*). By the freezing-point method of the *B.P.* as used for eucalyptol, oil of eucalyptus contains not less than 70% w/w of cineole. Sp. gr., 0.910 to 0.930; α_D , -5° to $+5^\circ$; n_{D20} , 1.458 to 1.470. Soluble in 5 volumes of alcohol (70%, sp. gr. 0.8896 to 0.8901). The *U.S.P. XII* requires the congealing point of Oleum Eucalypti to fall not below -15.4° , corresponding to not less than 70% of eucalyptol. Essence d'Eucalyptus, *Fr. Cx.* 1937, contains not less than 80% of eucalyptol. Oleum Eucalypti, *P. Helv. V*, determined by crystallisation of 5 ml. of the oil and 10 ml. of a 50% solution of resorcinol, decomposition of the separated crystals with sodium hydroxide solution and measurement of the cineole, contains 80 to 85% of cineole.

Eucalyptol (*B.P.*). $C_{10}H_{18}O=154.1$. By determination of the f.p. of a mixture of 3 g. of the dried substance and 2.1 g. of *o*-cresol,

a content of not less than 97.5% *w/w* of cineole, $C_{10}H_{18}O$, should be indicated. Sp. gr., 0.928 to 0.930; α_D , -1° to $+1^\circ$; n_{D20}^{20} , 1.456 to 1.460. F.p., not below 0° . Eucalyptol, *U.S.P. XII*, distills between 174° and 177° ; α_D at 25° , not more than $\pm 3^\circ$; n_{D20}^{20} , 1.4550 to 1.4600. Complies with tests for phenols.

Commercial Eucalyptus Oils. There are about ten eucalyptus oils met with in commerce, of which about seven are common. Their composition is very variable.

| Species | Principal Constituents |
|--|---|
| <i>E. polybractea</i> | Cineole (77 to 84%), pinene. |
| <i>E. dives</i> (type) | Piperitone (40 to 50%), phellandrene. |
| <i>E. phellandra</i> (<i>amygdalina</i>) | Cineole (20 to 35%), pinene, terpineol, phellandrene. |
| <i>E. Australiana</i> | Cineole (68 to 72%), pinene, terpineol. |
| <i>E. elaeophora</i> | Cineole (65 to 76%), pinene. |
| <i>E. sideroxylon</i> | |
| <i>E. leucoxylon</i> | |
| <i>E. citriodora</i> | |
| <i>E. Cneorifolia</i> | |
| <i>E. dives</i> var. "C" | Citronellal (70 to 85%). |
| <i>E. Macarthuri</i> | Cineole (69 to 73%), and terpenes. |
| | Cineole (60 to 75%), terpineol and terpenes. |
| | Geranyl acetate (60 to 72%), geraniol, eudesmol. |
| <i>E. radiata</i> | Phellandrene in abundance, and piperitol. |
| <i>E. Considemiana</i> | Cineole (50 to 70%), pinene, phellandrene. |
| <i>E. phlebophylla</i> | Pinene and eudesmol. |

Short monographs on the above are included, giving source, physical constants and composition. The oil of *E. Australiana* is sold in two fractions as "first hour oil" and "second hour oil." The former, which is the first oil obtained in the distillation, contains 70 to 82% of cineole, the latter contains only 25 to 40%. The author states that the oil has "no superior in aroma, flavour, etc., and is undoubtedly the finest eucalyptus oil for medicinal purposes." The oil of *E. phellandra* is also collected and sold as "first" and "second hour" oils. Methods for the determination of cineole by Kleber and von Rechenberg's process and by the ortho-cresol method, as given in the report of the Essential Oil Sub-Committee on Uniformity of Analytical Methods (*Analyst*, 1927, 276), were found to give close agreement. A qualitative and also a quantitative test for piperitone in *E. dives* oil is given, and also a quantitative test for citronellal in *E. citriodora* oil, and for the esters in *E. Macarthuri* oil.—A. R. Penfold, *Bull. tech. Mus., Sydney*, No. 2.

A number of physiological forms of *E. dives* exists yielding oils of widely different kinds:—

| Oil | Piperitone | Cineole | Phellandrene |
|------------------------|--------------|-----------|--------------|
| <i>E. dives</i> (type) | 46 to 53% | absent | present |
| " var. "A" | about 5% | | " |
| " var. "B" | 10 to 20% | 25 to 45% | " |
| " var. "C" | less than 5% | 60 to 75% | absent |

—*J. roy. Soc., N.S.W.*, 1929, 61, 54.

A very complete description of the eucalypts, in which over 170 species are examined, is given in "A Research on the Eucalypts especially in regard to their Essential Oils," by R. T. Baker and H. G. Smith, *Bull. tech. Mus., Sydney*, No. 24.

The physical and chemical constants for the oil of *Eucalyptus Australiana* are sp. gr., $15^\circ/15^\circ$, 0.9078; α_D , -11° ; n_{D20}^{20} , 1.4696. Solubility in 70% alcohol (by weight), 1.3 vols.; cineole, 50%; phellandrene, present in quantity; saponification no., 7.2; saponification no. after acetylation, 65.3.—A. R. Penfold and F. R. Morrison, *Perfum. essent. Oil Rec.*, 1936, 18.

Optically pure *l*- α -phellandrene has been prepared having sp. gr. 0.8324, refractive index 1.4724, and specific rotation 177.4 , all taken at 20° .—Hancock and Jones, per *Chem. & Drugg.*, i/1940, 215.

The *Rideal-Walker coefficients* of the principal eucalyptus oils.

The following tables appear in an article on "An investigation into the germicidal powers and capillary activities of certain Essential Oils," by S. Rideal, E. K. Rideal and A. Sciver (*Perfum. essent. Oil Rec.*, 1928, 285).

COMMERCIAL EUCALYPTUS OILS

| Crude Oil | R.-W. Co- effi- cients | Active Constituents | |
|--------------------------|---------------------------------|--|--|
| | | Principal | Minor |
| <i>E. polybractea</i> .. | 5 | Cineole 85% .. | Small amounts of the aromatic aldehydes. |
| <i>E. australiana</i> .. | 5 | Cineole 62% .. | Terpineol, geraniol, citral. |
| <i>E. dives</i> .. | 8 | Piperitone 52% .. | Piperitol. |
| <i>E. radiata</i> .. | 10 | Piperitol 20% .. | Piperitone. |
| <i>E. citriodora</i> .. | 8 | Citronellal 90% .. | |
| <i>E. cneorifolia</i> .. | 7.5 | Cineole 50% .. | Australol, cymene, aromatic aldehydes. |
| <i>E. phellandra</i> .. | 6 | Cineole 50% .. | Piperitone? |
| <i>E. Staigeriana</i> .. | 11 | Citral .. | Unidentified alcohol. |
| <i>E. Macarthuri</i> .. | 1 | Geranyl acetate 75% .. | Geraniol. |
| <i>E. hemiphloia</i> .. | 7 | Cymene, aromatic aldehydes, australol. | |

PURE CONSTITUENTS (FRESHLY PREPARED) OF EUCALYPTUS OILS

| Constituent | Nature | Source | R.-W. Coeffi- cients |
|--------------------------------|--------------------------|--|----------------------------|
| Cymene .. | hydrocarbon .. | <i>E. lactea</i> .. | 8 |
| Cineole .. | oxide .. | <i>E. polybractea</i> .. | 3.5 |
| Pinene, l.a. .. | terpene .. | <i>E. phlebophylla</i> .. | 1 |
| Phellandrene .. | terpene .. | <i>E. radiata (numerosa)</i> .. | |
| Limonene .. | terpene .. | <i>E. Staigeriana</i> .. | 1 |
| Aromadendrene .. | sesquiterpene .. | <i>E. nova-anglica</i> .. | under 1 |
| Terpineol .. | alcohol .. | <i>E. australiana</i> .. | 7.5 |
| Geraniol .. | alcohol .. | ex. geranyl acetate from <i>E. Macarthuri</i> .. | 21 |
| Piperitol .. | alcohol .. | <i>E. radiata (numerosa)</i> .. | 13 |
| Eudesmol .. | sesquiterpene alcohol .. | <i>E. Moorei</i> .. | under 1 |
| Geranyl acetate (85%) .. | ester .. | <i>E. Macarthuri</i> .. | none |
| Citral .. | aldehyde .. | <i>E. Staigeriana</i> .. | 19.5 |
| Citronellal .. | aldehyde .. | <i>E. citriodora</i> .. | 13.5 |
| Cuminal .. | aldehyde .. | <i>E. cneorifolia</i> .. | 12.75 |
| Phellandral .. | aldehyde .. | <i>E. cneorifolia</i> .. | 9.25 |
| Cryptal .. | aldehyde .. | <i>E. cneorifolia</i> and <i>E. hemiphloia</i> .. | 12 |
| Isovaleric aldehyde .. | aldehyde .. | <i>E. Macarthuri</i> .. | 5 |
| Piperitone .. | ketone .. | <i>E. dives</i> .. | 8 |
| Piperitone 90% (commercial) .. | ketone .. | <i>E. dives</i> .. | 8 |
| Australol .. | phenol .. | <i>E. cneorifolia</i> .. | 22.5 |

OLEUM GERANII

Oleum Geranii (B.P.C.). Contains not less than 21% of ester, calculated as geranyl tiglate, $C_{15}H_{24}O_2$. Sp. gr., 0.895 to 0.905 (French) or 0.894 to 0.904 (Algerian) or 0.888 to 0.896 (Bourbon); α_D , -7° to -11° (French), or -7° to -12° (Algerian) or -8° to -14° (Bourbon); n_{D20}° , 1.465 to 1.470 (French) or 1.465 to 1.467 (Algerian) or 1.462 to 1.467 (Bourbon). Soluble in 3 volumes of alcohol (70%).

Geranium oil from S. Africa. Analytical characters given.—*Perfum. essent. Oil Rec.*, 1931, 102

For the method of cultivation of geraniums for essential oil, see *Perfum. essent. Oil Rec.*, 1932, 173.

Experimental cultivation in Calabria. Yields of oil given.—*Perfum. essent. Oil Rec.*, 1934, 9.

Oil which compares favourably with standard types of commercial geranium oil has been produced in Kenya from varieties of *Pelargonium*. The characters and analytical data are fully reported in *Bull. imp. Inst., Lond.*, 1934, 196.

OLEUM GRAMINIS CITRATI

Oleum Graminis Citrati (B.P.C.). Assayed by the B.P. method for aldehydes in Oleum Cinnamomi, by interaction of the neutralised oil in benzene solution with hydroxylamine reagent and titration with N/2 potash in alcohol (60%), it should contain not less than 75% w/w of citral, $C_{10}H_{16}O$. Sp. gr., 0.895 to 0.908; α_D , -4° to $+1^\circ$; n_{D20}° , 1.483 to 1.489. Soluble in 3 volumes of alcohol (70%), sometimes appearing opalescent in 10 volumes.

The oil is usually sold on its citral content and used for the preparation of citral. It should not be confused with true verbena oil which is obtained from *Lippia citriodora* (Fam. Verbenaceæ) and contains citral but is of much more delicate odour.

Spanish verbena oil is obtained from *Thymus hyemalis* (Fam. Labiatae).—*Perfum. essent. Oil Rec.*, 1912, 212.

OLEUM JUNIPERI

Oleum Juniperi (B.P.C.). Sp. gr., 0.870 to 0.890 (English) and 0.865 to 0.895 (Hungarian); α_D , $+1^\circ$ to -10° (English) and -5° to -15° (Hungarian); n_{D20}° , 1.476 to 1.479 (English) and 1.479 to 1.484 (Hungarian). Oleum Juniperi, U.S.P. XII, has a sp. gr. at 25° of 0.854 to 0.879; α_D at 25° , 0° to -15° and n_{D20}° , 1.4740 to 1.4840.

Continental juniper berry oils are very variable in quality, probably due to their being by-products in the manufacture of other preparations. Italian oil is generally considered the best Continental oil.

English juniper berry oil is pre-eminently superior.—*Perfum. essent. Oil Rec.*, 1915, 63.

The effect of age on the oil is to increase its specific gravity and render it less soluble.—*Perfum. essent. Oil Rec.*, 1914, 5.

The Continental oil known as juniper wood oil is composed very largely of terpenes and has very little true juniper odour.

OLEUM LAVANDULÆ

Oleum Lavandulæ (*B.P. Add. I*). Is obtained from *Lavandula officinalis* Chaix and contains 7 to 12% *w/w* (English) or not less than 35% *w/w* (foreign) of esters calculated as linalyl acetate, $C_{12}H_{20}O_2$. Sp. gr., 0.882 to 0.900 (English) or 0.883 to 0.895 (foreign); α_D , -3° to -10° (English and foreign oils); n_{D20° , 1.459 to 1.470 (English) or 1.459 to 1.464 (foreign). Soluble with not more than a slight opalescence in 4 volumes of alcohol (70%). Determined by saponification of the esters by the *B.P.* method. The *U.S.P. XII* requires Oleum Lavandulæ to contain not less than 30% of esters as linalyl acetate; sp. gr. at 25° , 0.875 to 0.888; α_D at 25° , -3° to -10° ; n_{D20° , 1.4590 to 1.4700. The volume of the oil should not diminish when shaken with water, a diminution indicating alcohol; a limit test for acetins is described by saponification of the alcohol (5%) solution.

Continental lavender oil differs from English oil in having a much higher ester content. The proportion of esters in Continental oil, 25 to 60%, is very variable, depending on locality, cultivation, method of distillation and other factors. The esters in English oil vary from 7 to 14%, depending on season.

For discussion on the identity of the linalyl esters, see Dalton, *Perfum. essent. Oil Rec.*, 1926, 432; Langlais and Goby, *Perfum. essent. Oil Rec.*, 1926, 520; and *Perfum. essent. Oil Rec.*, 1927, 47; also Parry, *Perfum. essent. Oil Rec.*, 1927, 8.

English lavender oil comes intermediate between French and spike oils in cineole content.—Cocking, *Perfum. essent. Oil Rec.*, 1921, 339.

The figure obtained by the ortho-cresol method is not a true estimate of the cineole in oils that contain esters, and this method is not applicable to the estimation of cineole in French lavender oil.—Reed, *Perfum. essent. Oil Rec.*, 1932, 340.

Oleum Lavandulæ Spicatæ (*B.P.C.*). Is obtained from *Lavandula latifolia* Vill. and contains, when determined by the *B.P.* method for free alcohols, not less than 30% of free alcohols calculated as linalol. Sp. gr., 0.900 to 0.920; α_D , -4° to $+6^\circ$; n_{D20° , 1.462 to 1.469. Soluble in three volumes of alcohol (70%) and six volumes of alcohol (65%).

The quality of Spanish spike lavender oil is affected by the method of distillation. Steam distillation yields an oil of lower solubility but of superior odour to that obtained by open-fire distillation. According to Bordas (*Perfum. essent. Oil Rec.*, 1927, 129) the open-fire method of distillation is almost universally employed in Spain, because the apparatus for steam distillation cannot be taken to the hills where the process is carried out. It is stated that though the physical data are important the odour of the oil is the criterion of quality, and that the range for the sp. gr. is 0.898 to 0.910.

Sage (*Perfum. essent. Oil Rec.*, 1927, 45) gives as a guide for Spanish spike oil the following figures:—Sp. gr. at 15.5° , 0.900 to 0.915; α_D at 25° , -5° to -10° ; n_{D20° , 1.464 to 1.466; solubility in 70% alcohol, 1 in 1.5 to 1 in 2.5; esters as linalyl acetate, 3 to 7%; total acetylisable, not less than 30%. See also Sage, *Perfum. essent. Oil Rec.*, 1923, 228, "An Analytical Symposium of the Spanish Essential Oils." French spike lavender oil differs very little from Spanish.

Lavandin Oil. In the districts of S. France where lavender and spike grow side by side, hybrids are formed from which lavandin oil is obtained on distillation. This oil is intermediate in odour value between true lavender and spike. It is generally sold on an ester content of 25%.

OLEUM LIMONIS

Oleum Limonis (*B.P. Add. I*). Contains not less than 4% *w/w* of aldehydes calculated as citral, $C_{10}H_{16}O$. Sp. gr.,

0.857 to 0.861; α_D , +57° to 65°; n_{D20° , 1.474 to 1.476. Limit of non-volatile residue, determined under specified conditions, of 2 to 3% w/w. The U.S.P. XII oil has a sp. gr. at 25° of 0.849 to 0.855; α_D at 25°, +57° to +65.6°; n_{D20° , 1.4742 to 1.4755. The first 10% of the distilled oil has a α_D not more than 5° less than that of the original oil and n_{D20° , 0.0010 to 0.0027 lower. Oleum Citri, *P. Helv. V*, assayed by addition of hydroxylamine hydrochloride solution neutralised to bromophenol blue, and titration with N/2 alcoholic potash, contains not less than 3.5% of citral. The *P.G. VI* requires the oil to be soluble clearly 1 in 12 in alcohol 90%, or to show only a little fluorescent matter, and to be free from fatty oils and paraffin.

The Food and Drug Administration of the U.S. Dept. of Agriculture defined oil of lemon for food purposes as the volatile oil expressed, without the aid of heat, from the fresh peel of the lemon (*Citrus limonia* Osbeck) with or without previous separation of pulp and peel. *Terpeneless oil of lemon*: oil of lemon from which all or nearly all the terpenes have been removed.—*S.R.A., F.D. No. 2, Rev. 5, Nov., 1936*.

Oil of lemon is obtained from fresh lemon peel by expression. This is accomplished either by pressing and absorbing the oil with a sponge by hand, or by the use of machines. It is anticipated that the machine process of extraction will supersede the sponge method (see *Chem. & Drugg.*, i/1929, 134, 234, 308, and *ibid.*, ii/1929, 289). It is stated that the machine-made oil is inferior in flavour to hand-pressed oil.

Method of preparation described. As a result of the standardisation of the oil to a content of 4% of citral, a large proportion of the oil exported from Sicily is doctored down with lemon terpenes to meet the minimum standard.—*Chem. & Drugg.*, ii/1927, 161.

The oil should not be exposed to light or air, and the presence of lemon juice also causes deterioration.—*Finnemore*.

An oil with an optical rotation exceeding +76° indicates that the oil has suffered deterioration; the acid value of the fresh oil is very low, increasing on keeping. Oil which has deteriorated gives a brown colour on shaking with hydrochloric acid.—*Chem. & Drugg.*, i/1928, 780.

Hydroxylamine process of estimating aldehydes and ketones including citral.—A. H. Bennett and F. K. Donovan, *Analyst*, 1922, 146; see also Report of the Essential Oil Sub-Committee on Uniformity of Analytical Methods, *Analyst*, 1934, 105.

For the determination of the end-point with solution of hydroxylamine, daylight or a daylight lamp is essential.—C. T. Bennett, *Pharm. J.*, i/1933, 150.

Californian lemon oil, probably owing to the method of preparation, has a low residue on evaporation.

- Oleum Limonis Deterpenatum (B.P.C.). Contains 40 to 50% w/w of citral. Sp. gr., 0.890 to 0.905; α_D , -4° to -9°; n_{D20° 1.479 to 1.483. Soluble in 3 volumes of alcohol (80%).

Terpeneless oil of lemon is mainly produced in S. Italy and Sicily. The lemon oil used is the finest obtainable. The yield is about 5% with the following characteristics:—

Sp. gr., 0.897 to 0.904; n_{D20° , 1.477 to 1.483; α_D , -2.0° to -3.5°; citra content, 40 to 50%; solubility, 1 in 1 of 80% alcohol.

Citral obtained from lemon grass oil, if purified to such an extent that the verbena odour is removed, may be used as an adulterant as can be seen from the following figures:—

| | Sp. gr. | α_D | Citral % |
|-----------------------------|----------------|--------------|----------|
| Sesquiterpeneless lemon oil | 0.895 | 0 to -1 | 65 |
| Terpeneless lemon oil .. | 0.897 to 0.904 | -2.0 to -3.5 | 40 to 50 |
| Citral | 0.896 | 0 | 100 |

The ester content is a guide to the detection of citral adulteration, but flavour and aroma are the best guides in conjunction with the analytical data.—Dalton, *Perfum. essent. Oil Rec.*, 1928, 7.

The removal of the sesquiterpenes from the oil causes it to lose its sweetness and softness, and the best oil for flavour and aroma is one containing under 40% of citral.—E. J. Parry, *Chem. & Drugg.*, ii/1913, 378.

The fluorescence under ultra-violet light.—See note under *Oleum Aurantii*.

OLEUM MELALEUCÆ ALTERNIFOLIÆ

Tea Tree Oil. Commercial tea tree oil is obtained from *Melaleuca alternifolia*, *Melaleuca linariifolia* and *Leptospermum citratum*. The oil of *M. alternifolia*, which is also known as titrol, has the following characters: Sp. gr., 0.8950 to 0.9050; α_D , $+6.8^\circ$ to $+9.8^\circ$; $n_{D_{25}^\circ}$, 1.4760 to 1.4810; ester number, 2 to 7; ester number after acetylation, 80 to 90; cineole, not more than 10%. *M. linariifolia* yields a similar oil, but has a cineole content of from 20 to 45%. The oil of *L. citratum* differs entirely from the oils of *Melaleuca* and contains 85% of citral and citronellal.—A. R. Penfold, *Aust. J. Pharm.*, 1941, 450.

The oil of *Melaleuca alternifolia* should be distinguished from that of *Leptospermum citratum*, lemon scented ti-tree oil, which has the following characteristics: sp. gr., 0.8792 to 0.8856; α_D , $+3.5^\circ$ to 5.0° ; $n_{D_{20}^\circ}$, 1.4688 to 1.4757. Containing 75 to 85% of total aldehydes, principally citral (45 to 50%), citronellal about 35%.—*Bull. tech. Mus., Sydney*, also *Perfum. essent. Oil Rec.*, 1936, 159; *ibid.*, 1929, 155.

OLEUM NEROLI

Oleum Neroli (B.P.C.). Soluble in 2 volumes of alcohol (80%) becoming turbid with more alcohol. Sp. gr., 0.870 to 0.885; α_D , 0° to $+8^\circ$; $n_{D_{20}^\circ}$, 1.468 to 1.477. Saponification value, not more than 70. *Oleum Aurantii Florum*, *N.F. VII*, has a sp. gr. at 25° of 0.863 to 0.880; α_D at 25° , $+1^\circ 30'$ to $+9^\circ 8'$. Complies with the same solubility test.

Oleum Patchouli. An account of the cultivation and distillation of patchouli oil in the Seychelles.—Haines, *Perfum. essent. Oil Rec.*, 1935, 171.

OLEUM PINI AROMATICUM

Oleum Pini Aromaticum (B.P.C.). Soluble in 2 volumes of alcohol (70%). Sp. gr., 0.9325 to 0.9465; α_D , $+7^\circ$ to $+15^\circ$; $n_{D_{20}^\circ}$, 1.4790 to 1.4870. The chief constituents of the oil are tertiary alcohols, principally α -terpineol, of which up to 70% may be present, secondary alcohols, mainly borneol and fenchyl alcohol, and smaller amounts of methyl chavacol and phenolic ethers.

OLEUM PINI PUMILIONIS

Oleum Pini Pumilionis (B.P.C.). Contains not less than 4% w/w of esters, calculated as bornyl acetate, $C_{12}H_{20}O_2$. Sp. gr., 0.865 to 0.873; α_D , -6° to -15° ; n_{D20} , 1.470 to 1.480. The U.S.P. XII oil is required to contain not less than 4% of esters as bornyl acetate; soluble in 4.5 to 8 volumes of alcohol (90%); sp. gr. at 25° , 0.853 to 0.869; α_D at 25° , -5° to -15° . Less than 4% of the oil should distil below 165° .

The limits as set forth in the literature for the physico-chemical properties should be revised in accordance with the following determined on genuine samples:—sp. gr. at 25° , 0.853 to 0.871; optical rotation, -3° to -16° ; refractive index at 20° , 1.4750 to 1.4800; ester content (calculated as bornyl acetate) 3 to 10%; solubility at 25° , 1 in 4.5 to 10 parts of alcohol 90%, often with turbidity; boiling range, less than 10° , distills below 165° .—C. H. Grimm, E. E. Langenau and E. S. Guenther, *J. Amer. pharm. Ass., Sci. Edn.*, 1941, 209.

OLEUM ROSÆ

Oleum Rosæ (B.P.C.). Should congeal at 18° to 22° and melt at 19° to 23° , when tested by the method of the B.P. for Oleum Anisi. Sp. gr. ($30^\circ/15.5^\circ$), 0.852 to 0.862; α_D , -2° to -4° ; n_{D25} , 1.458 to 1.465. Oleum Rosæ, U.S.P. XII, produces a clear mixture with an equal volume of chloroform; on diluting with 20 volumes of alcohol (90%) the liquid is neutral or slightly acid to litmus, and on standing produces a crystalline deposit; sp. gr. ($30^\circ/15^\circ$), 0.848 to 0.863; α_D , -1° to -4° at 25° ; n_{D30} , 1.457 to 1.463.

Analytical Characters of Bulgarian Otto of Rose. Ethyl alcohol is stated to be a natural constituent of the oil; it should therefore not be washed before analysis. The stearoptenes content is very variable. The acid value varies from 1.4 to 3.8. A high refractive index and a high rhodinol content appear to be characteristic of good Bulgarian otto. Physical and analytical data given for a number of samples.—Garnier and Sabétay, *C. R. Acad. Sci., Paris*, 1933, 1748; see also *Perfum. essent. Oil Rec.*, 1934, 347. Any oil with less than 40% of rhodinol is suspect.

The presence of ethyl alcohol is doubtful and may be due to ethoxy, methoxy or other groups interfering with the Zeisel reaction. The ratio of citronellol to geraniol is a standard rather than the individual percentages of those constituents. Table of physical and analytical data with ratio $\frac{\text{citronellol}}{\text{geraniol}}$ is given.—Parry and Seager, *Perfum. essent. Oil Rec.*, 1934, 213. See also *Perfum. essent. Oil Rec.*, 1933, 149, and Garnier, *Perfum. essent. Oil Rec.*, 1933, 370; also *Perfum. essent. Oil Rec.*, 1936, 278.

Details of cultivation and analysis of small samples of oil of rose in Cyprus.—*Perfum. essent. Oil Rec.*, 1930, 257.

GERANIOL $C_{15}H_{26}O=154.144$ (three-quarters of the liquid portion), and citronellol $C_{15}H_{26}O=156.16$ (the remaining 25%). Linalol is isomeric with geraniol, sp. gr., 0.870, B.p., 197° . It is contained in coriander, thyme and other oils and is either + or - rotatory.

Geraniol is an important base in perfumery. It is not made synthetically, but occurs in a number of oils, especially palmarosa and Java citronella—in these it is free, in other oils it occurs as ester: acetate and tiglate.

"RHODINOL" is a blend of the two alcohols geraniol and citronellol, from pelargonium leaf oil. Some workers give the name as synonymous with geraniol—others as synonymous with citronellol.

75% or 76% at most is the highest amount of alcohol calculated as geraniol that should be allowed in a normal pure otto. Pure otto never has specific

gravity as high as 0.862. Frequently it is as low as 0.850. Any otto with a refractive index below 1.4600 is adulterated, and almost invariably with alcohol. Considering that about 50% of the adulterated samples contain alcohol, which is used to adjust the high sp. gr. and refractive index of the geraniol compounds added, the following test is valuable:—

If 5 ml. be well shaken with 10 ml. of warm water and the mixture allowed to separate, the refractive index of the washed oil at 25° should not differ from that of the original oil by more than 0.0015 (absence of alcohol).

The determination of the refractive index should be made on the separated otto when quite clear, filtered if necessary, but not dried with any drying agent, since the original oil, owing to the method of distillation, is saturated with water.—Parry.

Although the predominating constituent, geraniol is by no means the most important, as both citronellol and nerol, and esters of the respective alcohols and other bodies, contribute largely to the fragrance of the oil. Phenyl ethyl alcohol, which possesses a mild odour, appears to be contained in otto and in neroli oil, not only as such, but also in the form of esters of benzoic and phenyl acetic acids. Although this alcohol is contained in exceedingly small quantity in otto, it represents quantitatively the chief volatile constituent of rose petals. Being freely soluble in water, it remains behind for the most part in the aqueous portion of the distillate from which the otto has been removed.

The so-called stearoptene of otto is a mixture of homologous hydrocarbons.

OLEUM ROSMARINI

Oleum Rosmarini (*B.P. Add. II*). Contains not less than 2% *w/w* of esters as bornyl acetate, $C_{12}H_{20}O_2$, and not less than 9% *w/w* of free alcohols as borneol, $C_{10}H_{18}O$. Sp. gr., 0.900 to 0.919; α_D , -5° to $+10^\circ$; n_{D20° , 1.466 to 1.476. Soluble in 1 volume of alcohol (90%) and in 10 volumes of alcohol (80%). Oleum Rosmarini, *U.S.P. XII*, contains not less than 1.5% of esters as bornyl acetate, and not less than 10% of total borneol, free and as esters. Sp. gr. at 25°, 0.894 to 0.912; α_D at 25°, -5° to $+10^\circ$; n_{D20° , 1.4640 to 1.4760.

Spanish rosemary oil of really first quality can scarcely be distinguished from French oil, but the coarser quality is, as a rule, found on the market. For analytical characters see Maurice and Salamon, *Perfum. essent. Oil Rec.*, 1923, 231, also Simmons, *Perfum. essent. Oil Rec.*, 1923, 233.

The ester content of Spanish oil is usually below the *B.P.* limit.

For the characters of Sicilian essential oils, including rosemary, see Pellini, *Chem. & Drugg.*, ii/1923, 391, and for Yugoslavian oils see *Perfum. essent. Oil Rec.*, 1931, 62.

The presence of light camphor oil may be detected by the test for furfuraldehyde, *vide* test under *Ol. Menth. Pip.*

OLEUM SANTALI

Oleum Santali (*B.P. Add. I*). Contains not less than 2% *w/w* of esters, calculated as santalyl acetate, $C_{17}H_{26}O_2$, and not less than 90% *w/w* of free alcohols, calculated as santalol, $C_{15}H_{24}O$. Sp. gr., 0.973 to 0.985; α_D , -15° to -20° ; n_{D20° , 1.505 to 1.510. Soluble at 20° in 5 volumes of alcohol (70%). The *N.F. VII* oil has a sp. gr. at 25° of 0.965 to 0.980; α_D , -15° to -20° at 25°; n_{D20° , 1.500 to 1.510; yields not less than 90% of alcohols, as santalol.

Structure of the woods of sandalwood substitutes described.—*Perfum. essent. Oil Rec.*, 1931, 332. Kalamet, an Indian substitute.—*Perfum. essent. Oil Rec.*, 1932, 318.

Analytical characters of East Indian oil.—Clevenger, *Quart. J. Pharm.*, 1932, 590.

West Indian sandalwood oil is obtained from the wood of *Amyris balsamifera*. The oil is dextrorotatory. It contains amyrol, probably a mixture of alcohols which differ from santalol.

The yield of oil was found to vary considerably, being least in sandalwood chips, probably due to the inclusion of larger proportions of sap wood. The sp. gr. is sometimes outside the U.S.P. XI limits (0.965 to 0.980 at 25°), values both above and below being found. The optical rotation gave values up to -22.50. The refractive index of official and non-official varieties of sandalwood oil are essentially identical. The sp. gr. and refractive index are of little value for detecting non-official varieties, i.e., Australian, New Caledonian, African and *Amyris* species but the optical rotation is important.—J. F. Clavenger, *J. Amer. pharm. Ass.*, 1938, 580.

Oleum Santali Australiensis (B.P.). Contains not less than 90% w/w of free alcohols as $C_{15}H_{24}O$. Sp. gr., 0.970 to 0.976; α_D , -3° to -10°; n_{D20} , 1.498 to 1.508. Soluble in 3 to 6 volumes of alcohol (70%). Essence de Santal d'Australie and Essence de Santal Citrin, *Fr. Cx.* 1937, contain 80% of santalol, by acetylation in presence of pyridine.

Numerous papers have been published comparing East Indian and Australian sandalwood oils.

West Australian sandalwood oil of good quality is now available, containing 95% alcohol (calculated as santalol) and closely resembling the East Indian oil, although it has not been proved that the alcohols are identical with the santalols of the East Indian oil. The oil, which is obtained from *S. spicatum* and *S. lanceolatum*, is not inferior therapeutically to that obtained from *S. album*.—P. May, *Pharm. J.*, i/1928, 368.

No evidence that the alcohols in W. Australian oil differ from those of Mysore oil, and its therapeutic value is at least equal to that of *S. album*.—W. H. Simmons, *Chem. & Drugg.*, i/1928, 171. See also a *contra* view.—A. R. Penfold, *Chem. & Drugg.*, ii/1928, 496.

See also Venkatesaiya and Watson, *J. Soc. chem. Ind., Lond.*, Nov. 1928; Penfold, *Perfum. essent. Oil Rec.*, 1928, 417; and 1933, 46; also *ibid.*, 1932, 349, 376.

For the volatile oil of Queensland sandalwood (*Santalum lanceolatum*), see Jones and Smith, *Perfum. essent. Oil Rec.*, 1931, 47.

For the therapeutic action of sandalwood oils, both Australian and East Indian, see Boldecker and Ludwig, *Pharm. Ztg., Berlin*, 1928, 73, 938, per *Quart. J. Pharm.*, 1928, 666. Australian oil has no antiphlogistic properties and its substitution for the oil of *Santalum album* is not permissible.

Determination of Free Alcohols by means of acetic anhydride and sodium acetate includes free alcohols and combined, but gives deficient results for tertiary alcohols such as linalol. The usual method of acetylation gives results some 10% higher than those obtained in the presence of pyridine or by phthalic esterification; it is known to include also alcohols combined as esters, but when allowance is made for these the results are still from 5 to 8.5% higher. Method using pyridine in the process of acetylation, see Delaby, Sabetay and Breugnot, *Perfum. essent. Oil Rec.*, 1935, 334.

OLEUM SASSAFRAS

Oleum Sassafras (B.P.C.). Soluble in 3 volumes of alcohol (90%) the solution being neutral to litmus. Sp. gr., 1.070 to 1.084; n_{D20} , 1.523 to 1.531; α_D , +1° to +5°. Oleum Sassafras, U.S.P. XII, should be soluble in 2 volumes of alcohol

(90%). Sp. gr. at 25°, 1.065 to 1.077; α_D at 25°, +2° to +4°; n_{D20° , 1.5250 to 1.5350.

Safrolum (B.P.C.). $C_{10}H_{10}O_2=162.1$. The m.p., determined by the B.P. method for Oleum Anisi, is not below 11° and the congealing point not below 10°. Sp. gr., 1.104 to 1.107; n_{D20° , 1.536 to 1.539. Soluble in 3 volumes of alcohol (90%) and in 10 volumes of alcohol (80%).

OLEUM TEREBINTHINÆ

Oleum Terebinthinæ (B.P.). Sp. gr., 0.860 to 0.870; n_{D20° , 1.467 to 1.477. Iodine value, using a larger quantity of iodine monochloride and allowing absorption to proceed for exactly one hour, not less than 340. Residue, by rapid evaporation from a flat dish on a water-bath, not more than 0.5%. The B.P. Add. I directs that in determining the iodine value of Oleum Terebinthinæ the oil be weighed into a small glass tube and introduced into a dried vessel containing the iodine monochloride solution and carbon tetrachloride. Absorption is allowed to proceed in the dark for exactly 1 hour at a temperature of 15° to 20°. The U.S.P. XII oil has a sp. gr. at 25° of 0.854 to 0.868. n_{D20° , 1.4680 to 1.4780. 90% of the oil distils between 154° and 170°; complies with a test for acidity, and a test for mineral oil, when the unabsorbed viscous layer, on mixing with 4 volumes of sulphuric acid at 60° to 65°, does not exceed 1%; 2% w/v of residue on evaporation is allowed. The U.S.P. XII specifies that Oleum Terebinthinæ Rectificatum be used for internal use and it must not have become turbid; sp. gr. at 25°, 0.853 to 0.862; non-volatile residue, not more than 0.3%.

Terebinthina (N.F. VII). Contains not more than 2% of foreign organic matter; alcohol-insoluble residue, not more than 2%.

The B.P. specifies "oil distilled from the oleo-resin"; the oil so obtained is commercially known as "gum turpentine" to distinguish it from "wood turpentine" which is the first fraction obtained on the steam distillation of the stumps and waste. The two varieties closely resemble one another. For distinction between gum and wood turpentine see Parry, *The Chemistry of Essential Oils and Artificial Perfumes*.

The optical rotation of turpentine derived from different species of Pinus compared.—Herty, *Yearb. Pharm.*, 1908, 199.

Examination of turpentine and turpentine substitutes. Wood turpentine may be detected by shaking with an equal volume of saturated sulphurous acid solution. Wood turpentine assumes a yellow colour, Russian and Swedish turpentine become greenish-yellow, and American and French turpentine remain colourless.—J. H. Coste, *Analyst*, 1908, 219.

A rapid method for the determination of petroleum in turpentine is given by Frey, *Svensk farm. Tidsskr.*, 1908, per *Quart. J. Pharm.*, 1908, 201.

Assay. The following assay and standard for turpentine in Liniment of Turpentine is recommended by the Sub-Committee on the Assay of Galenicals of the Committee on Pharmaceutical Chemistry:—Weigh accurately about 50 g., acidify with dilute sulphuric acid, using solution of methyl orange as indicator, distil in steam and collect the distillate in a separator; from time to time separate the aqueous portion of the distillate and return it to the boiling-flask; when all the volatile matter has been distilled, remove the aqueous portion of

the distillate and measure the oily liquid. The oily liquid obtained should amount to not less than 63% *v/w* and not more than 70% *v/w* and should have refractive index at 20°, 1.465 to 1.477.—(*British Pharmacopœia Commission Report*, No. 11, May 1939.)

Turpentine and White Spirit for Paints.

The following *British Standard Specifications* have been prepared by the British Standards Institution for turpentine and white spirit for use in paints:—

B.S.S. No. 244—1936 relates to turpentine type I, and includes requirements regarding description (genuine refined gum spirits of turpentine), sp. gr. (0.862 to 0.872 at 15.5°), distillation (not more than 1% below 150° and not less than 95% below 170° at 760 mm. pressure), non-volatile residue (not exceeding 2% and is wholly organic), refractive index (1.469 to 1.478), unpolymerisable residue (not more than 11%), flash point (not below 90°F., Abel's closed test) and sampling.

B.S.S. No. 290—1936 refers to turpentine type II, which is genuine turpentine distilled from pine oleo-resins or obtained from resinous wood by steam or destructive distillation; the specifications give requirements for specific gravity (0.859 to 0.875), distillation (as for type I except 70% below 170° and not less than 90% below 180°), non-volatile residue (2%), refractive index (1.463 to 1.483), unpolymerisable matter (16%), flash point and sampling.

B.S.S. No. 245—1936 covers the requirements of white spirit for use in thinning paints and varnishes. It should be wholly a petroleum product complying with requirements for distillation (not more than 10% below 150° and not less than 80% below 190°), freedom from grease, non-volatile residues (on water-bath and by spontaneous evaporation), neutrality, freedom from objectionable sulphur compounds, volatility, and flash point (not below 78°).

The appendices to the three specifications give the methods to be used for determining the volatility, freedom from objectionable sulphur compounds, the polymerisation test and the procedure for the distillations.

Lævo-pinene or terebentene of Berthelot is obtained by fractionation of French oil of turpentine as a colourless mobile liquid of characteristic odour. Sp. gr., 0.8767 at 0° and 0.8619 at 17.9°.

Russian Turpentine Oil. Authentic samples contain 40 to 70% distilling between 155° and 160°, and consisting chiefly of pinene. The oils arriving in the London markets have these "middle runnings" removed.

Indian Turpentine, from *P. longifolia*, constituents of.—J. L. Simonsen, *J. chem. Soc.*, May 1929, 570.

OREGON AND COLORADO DOUGLAS FIR OILS from trees grown in Britain. Geraniol the chief odorous constituent of the first. The odour of the other appears due to the large proportion of pinene and bornyl acetate.—C. T. Bennett, *Perfum. essent. Oil Rec.*, 1920, 218.

Terebenum (B.P.). Sp. gr., 0.862 to 0.87; α_D , -2° to $+2^\circ$; n_{D20° , 1.471 to 1.474. Distils between 160° and 190° leaving only a slight viscous residue, and not less than 80% distils between 165° and 185°. Residue by rapid evaporation from a flat dish on a water-bath, not more than 2% *w/w*.

Terpini Hydras. (B.P.C.) melts between 116° and 119°. Ash, not more than 0.05%.

Terpineol (B.P. Add. VI). $C_{10}H_{18}O = 154.1$. Sp. gr., 0.934 to 0.938; n_{D20° , 1.4825 to 1.4855. Not less than 96% distils between 214° and 220°.

OLEA EXPRESSA

The term "expressed," as applied to oils, is only of generic value, as a great number of these oils are not prepared commercially

by an expression process. The methods used may be divided into three classes, a process of boiling out by water or steam, expression, and extraction by volatile solvents. The first two of these methods are very old, and in various parts of the world they are still practised in what is probably their original and very crude manner. Oils obtained by any of the above methods are often further treated by a refining and bleaching process to improve their quality. A **boiling out process** is mostly used for the preparation of animal oils and fats; the crude material being boiled with water or salt solution and the oil separated by gravity; oils obtained in this way are generally subjected to a refining process. **Expression** is used for a very large number of vegetable oils. The presses used in some parts of the world are still very crude, consisting of a lever and weights; in modern machinery advantage is taken of the hydraulic press, in which pressure round about three tons per square inch may be exerted. When the oil is expressed from the whole or ground material without any application of heat it is called "cold-drawn." Very often the ground material is subjected to moderate heating, with or without the addition of water, in the form of steam, and then pressed. In some cases these processes may be combined, a portion of the oil being obtained by cold expression and the material being then ground, heated and again pressed. Oils obtained by a process involving heating are generally darker in colour and of inferior taste and odour. **The solvent extraction process** is used for many different oils and also to remove the oil left in the "cake" by the hydraulic presses. Various solvents are used, petroleum ether, carbon tetrachloride, carbon disulphide and some of the chloro derivatives of ethane and ethylene. The product is generally inferior to an expressed oil, may contain traces of solvent or non-volatile residues, and generally requires refining and bleaching. **Refining and bleaching processes** are applied to oils to remove excessive fatty acids, colouring matter, mucilaginous and albuminous matter and the products of rancidity. The general process is first to neutralise the oil with soda, the free fatty acid being thus removed as soap. The oil is then, if necessary, bleached by charcoal or absorbent earths, and finally treated by steam *in vacuo* to remove traces of volatile substances. Details of the method of extraction used for industrial oils, with the chemical components and analytical data, can be found in *Chemical Technology and Analysis of Oils, Fats and Waxes*, Lewkowitsch; *Laboratory Companion to Fats and Oils Industry*, Lewkowitsch; *Edible Oils and Fats*, Fryer and Weston; *Technical Handbook of Oils, Fats and Waxes*, Elsdon; and other standard works.

British Standard Specifications have been prepared by the Technical Committee of the British Standards Institution for a number of animal and vegetable oils. Certain of these oils have monographs in the *B.P.* and *B.P.C.*, in which cases the British Standard Specifications differ in certain particulars and are not intended to be applied to medicinal oils. The following have been published:—

| | B.S.S. | Date |
|---|--------|------|
| Castor oil, first quality | 650 | 1936 |
| Coconut Oil | 628 | 1935 |
| Cod-liver Oil for sulphonation purposes | 868 | 1939 |
| Controlled Cod-liver Oil mixtures for animal feeding purposes | 910 | 1940 |
| Cotton Seed Oil, refined | 655 | 1936 |
| Ground Nut Oil | 629 | 1935 |
| Japanese and/or Korean Sardine Oil | 783 | 1938 |
| Linseed Oil, raw, for general purposes | 632 | 1935 |
| Linseed Oil, for paints | 242 | 1936 |
| Maize Oil, crude | 651 | 1936 |
| Oils, Thinners, Driers and Extenders for paints | 925 | 1940 |
| Olive Oil | 630 | 1935 |
| Palm Kernel Oil, crude | 652 | 1936 |
| Perilla Oil | 654 | 1936 |
| Rape Seed Oil | 631 | 1935 |
| Sesame Oil | 656 | 1936 |
| Soya Bean Oil | 653 | 1936 |
| Tung Oil, Type F for paints | 391 | 1936 |
| Veterinary Cod-liver Oil | 839 | 1939 |
| Vitamins A and D in Oil for animal feeding purposes | 909 | 1940 |
| Whale Oil | 836 | 1939 |
| Sampling of fats and fatty oils in packages or in bulk | 627 | 1935 |

Determination of Iodine Values of Oils and Fats. The iodine value of a substance is the weight of iodine absorbed by 100 parts by weight of the substance when determined by one of the following methods (*B.P. Add. II*):—

(1) **Iodine Monochloride Method.** The iodine monochloride solution is prepared by one of the following methods: (a) Dissolve 13 g. of iodine in 1000 ml. of glacial acetic acid. To 20 ml. of this solution add 15 ml. of solution of potassium iodide and 100 ml. of water and titrate the solution with N/10 sodium thiosulphate. Pass chlorine, washed and dried, through the remainder of the iodine solution until the amount of N/10 sodium thiosulphate required for titration is approximately, but not more than, doubled. (b) Iodine trichloride, 8 g.; iodine 9 g.; glacial acetic acid, sufficient to produce 1000 ml. Dissolve the iodine trichloride in about 200 ml. of glacial acetic acid; dissolve the iodine in about 500 ml. of glacial acetic acid; mix the two solutions, and add sufficient glacial acetic acid to produce the required volume.

Place the oil, or fat, accurately weighed, in a dry stoppered vessel, add 10 ml. of carbon tetrachloride, and dissolve. Add 20 ml. of solution of iodine monochloride, insert the stopper, previously moistened with solution of potassium iodide, and keep in a dark place at a temperature of about 17° for half an hour. Add 15 ml. of solution of potassium iodide and 100 ml. of water; shake, and titrate with N/10 sodium thiosulphate, using mucilage of starch as indicator. Note the number of millilitres required (a). At the same time carry out the operation in exactly the same manner but without the substance being tested, and note the number of millilitres of N/10 sodium thiosulphate required (b).

Calculate the iodine value from the following formula:—

$$\text{Iodine value} = \frac{(b - a) \times 0.01269 \times 100}{\text{Weight (in grammes) of substance.}}$$

The approximate weight, in grammes, of substance to be taken may be calculated by dividing 20 by the highest expected iodine value. If more than half the available halogen is absorbed, the test must be repeated on a smaller quantity of the substance being tested.

(2) **Pyridine Bromide Method.** The pyridine bromide solution is prepared as follows: Dissolve 8 g. of pyridine and 10 g. of sulphuric acid in 20 ml. of pure glacial acetic acid, keeping the mixture cool. Add 8 g. of bromine dissolved in 20 ml. of pure glacial acetic acid and dilute to 1000 ml. with pure glacial acetic acid.

Place the substance, accurately weighed, in a dry, stoppered vessel, add 10 ml. of carbon tetrachloride and dissolve. Add 25 ml. of solution of pyridine bromide and allow to stand for 10 minutes in a dark place. Complete the determination as described under the iodine monochloride method, beginning with the words "Add 15 ml. . . .". The approximate weight in grammes of substance to be

taken may be calculated by dividing 12.5 by the highest expected iodine value. If more than half the available halogen is absorbed the test must be repeated on a smaller quantity of the substance being tested.

For description of the original method of Von Hübl, see p. 190, Vol. II, 21st Edn.

Dichloramine has been used for the determination of iodine values and the results, within the limits of experimental error, agree with those obtained with Wijs' solution. The reagent is prepared as follows:—To a solution of 0.05 mol. of dichloramine in about 700 ml. of halogen stable acetic acid add 0.1 mol. of dry powdered potassium iodide in small quantities at a time with continual shaking. When all the iodide is dissolved, dilute to 1000 ml. with acetic acid.

The reagent may be stored in a bottle with a dark felt cover to minimise decomposition by light and to deter freezing. The iodine equivalent is determined in the usual way by determining the iodine liberated from a known volume of excess of aqueous potassium iodide.

Comparison of results of the Hübl, Wijs, Hanus and Margosches methods by determining with them the iodine values of oleic, linolic and erucic acids, triolein and trilinolin. The methods of Hübl and Hanus show no significant differences for oleic acid and trilinolin, and methods of Wijs and Hanus show no significant differences with linolic acid, erucic acid and triolein.—I. Netto, per *Analyst*, 1936, 867.

THIOCYANOGEN VALUE—a new constant of oils and fats—is of special use in determining the amount of linolic acid in an oil. Thiocyanogen, like iodine, is quantitatively absorbed by substances containing a double bond, but with fatty acids containing a triple bond (stearolic, behenolic) there is no absorption; and with linolic acid there is only absorption of half the thiocyanogen corresponding to its iodine value.—*Analyst*, 1926, 157, 264.

Determination of Unsaponifiable Matter. Unsaponifiable matter is defined as consisting of that material present in oils and fats which, after saponification of the oil or fat by caustic alkali and extraction by a solvent specified remains non-volatile on drying at 80°.

The following is the method given in *B.P. Add. II* for the determination of unsaponifiable matter in fixed oils and fats.

Weigh accurately a quantity of oil or fat, not exceeding 2.5 g., but not less than 2.0 g., and boil on a water-bath for one hour with 25 ml. of N/2 alcoholic potassium hydroxide in a flask provided with a reflux condenser. Wash the contents of the flask into a separator by means of 50 ml. of water and extract by shaking vigorously, while still slightly warm, with three successive quantities of 50 ml. of ether, washing the flask with the first quantity of ether. Mix the ethereal solutions in a separator containing 20 ml. of water. (If the ethereal solutions contain solid suspended matter, filter them into the separator through a fat-free filter-paper and wash the filter-paper with ether.) Gently rotate the separator for a few minutes without violent shaking, allow the liquids to separate, and run off the aqueous layer. Wash the ethereal solution by shaking vigorously with two successive quantities of 20 ml. of water; then wash three times with 20 ml. of N/2 aqueous potassium hydroxide by shaking vigorously on each occasion, each alkali treatment being followed by washing with 20 ml. of water. Finally, wash with successive quantities of 20 ml. of water until the aqueous layer is no longer alkaline to solution of phenolphthalein. Transfer the ethereal extract to a weighed flask, washing out the separator with ether; distil off the ether and add 3 ml. of acetone. By the aid of a gentle current of air remove the solvent completely from the flask, which is preferably held obliquely and rotated, almost entirely immersed in a boiling water-bath. Dry to constant weight at a temperature not exceeding 80°. After attaining constant weight, dissolve the contents of the flask in 10 ml. of freshly-boiled alcohol (95%), previously neutralised to solution of phenolphthalein. Titrate with N/10 sodium hydroxide, using solution of phenolphthalein as indicator. If the amount of N/10 sodium hydroxide required does not exceed 0.1 ml., the unsaponifiable matter is to be taken as the amount weighed. Calculate the unsaponifiable matter as a percentage of the oil or fat. If the amount of N/10 sodium hydroxide required exceeds 0.1 ml. the test has not been properly carried out and must be repeated. *Reagent.*—Solution of potassium hydroxide, alcoholic, N/2; a solution containing 28.05 g. KOH in 1000 ml. of alcohol (95%).

Determination of Acetyl Value. The *B.P. Add. VI* defines the acetyl value of a substance as the number of mg. of potassium hydroxide required to

neutralise the acetic acid liberated when 1 g. of the acetylated substance is hydrolysed. The acetyl value may be calculated from the following formula:

$$\text{Acetyl Value} = \frac{(b - a) 1336}{1336 - a}$$

where a = saponification value of the original substance

and b = saponification value of the substance acetylated by the method described in the *B.P.* for the determination of free alcohols in volatile oils.

Ultra-violet Absorption of certain vegetable oils may be used as an indication of their commercial treatment. The mean value of e at 270.0 m μ ($e = \log. I_0/I$) for 1% solutions in hexane in a cell 1 cm. thick, for virgin olive oil = 0.160, for refined oil = 0.700 and for oil from refined pulps = 1.450. The effect of heating an oil is to raise e .—J. Guillot, *Ann. Falsif.*, 1935, 28, 69, per *Analyst*, 1935, 432.

Colour Measurement of Oils. The inaccuracies of the Lovibond method of measurement due to the variations of the human sight are eliminated by the use of Wratten filters to obtain a light in a narrow wave-band, a copper sulphate cell to eliminate the infra-red and a photo-electric cell to measure the light transmitted through a cell containing the oil. A series of Wratten filters may be used or the readings confined to two, Wratten No. 62 and No. 71A. The percentage of light transmitted is obtained by dividing the reading obtained with the oil by that obtained with an empty cell and multiplying by 100. This figure subtracted from 100 gives the percentage of light extinguished. Readings are reproducible to within 1 or 2% by different workers. The results obtained with a Wratten No. 71A are roughly correlated to those obtained in Lovibond red units but are in different units.—E. R. Bolton and K. A. Williams, *Analyst*, 1935, 447.

Products Responsible for the Taste and Odour of Vegetable Oils. The process of refining removes something which cannot be detected chemically, but which is sufficient to alter the taste of the oil. This occurs during the deodorisation with superheated steam. The substances removed during this process are hydrocarbons. In olive and arachis oils the amount of these substances is estimated at 0.07 and 0.0018 g. per kg. respectively.—Mercelet, *J. Pharm. Chim.*, ii/1936, 213.

Estimation of Rancidity. The Kreis test is usually employed. Kerr's modification of this test is as follows: 10 ml. of melted fat or oil is vigorously shaken for thirty seconds with 10 ml. of hydrochloric acid (sp. gr. 1.19), 10 ml. of a 0.1% solution of phloroglucinol in ether is added, and the mixture shaken. A red colour in the acid layer indicates that the fat or oil is rancid.—*Industr. Engng Chem., anal. Edn.*, 1918, 471.

The substance present in rancid oils and fats which gives rise to the red colour in the Kreis test is epihydrinaldehyde. The Kreis test is usually applied as a qualitative test, the quantitative result by measurement of the depth of the red colour being difficult and often inaccurate. The following method which is standardised on epihydrinaldehyde gives quantitative results. The oil (0.5 to 1 g.) is dissolved in sufficient of a 1% solution of phloroglucinol in acetone to give a 10% solution. A number of drops of sulphuric acid equal to the number of ml. of solution are added, and the mixture is placed in a water-bath at 10° for 15 minutes. The depth of the red colour is measured in a Lovibond tintometer. Red units $-0.625 \div 97$ = rancidity as percentage of epihydrinaldehyde. Water must be excluded from the test.—M. Pyke, *Analyst*, 1935, 515.

Fats may undergo degradation in other ways than by the formation of epihydrinaldehyde, the proportion of which increases to a certain point, but finally it may become constant or even diminish as auto-oxidation proceeds. Strict parallelism between the intensity of the Kreis reaction and the extent to which auto-oxidation has proceeded cannot therefore be expected. The red colour is produced by a number of substances such as allyl alcohol, allylamine and diallylcarbamide; also with advanced deterioration of fats sparingly soluble, colourless aldehyde phloroglucosides are formed, the Kreis colour reaction being prevented. The following method avoids the interference of any of these causes. The oil is mixed in a short test-tube with an equal quantity of ice-cooled concentrated hydrochloric acid. A cotton-wool plug, inserted in the upper dry part of the tube, is moistened with 1 ml. of a 0.1% solution of phloroglucinol in alcohol and 10 drops of at least 20% hydrochloric acid. The tube is well shaken for 1 to 2 minutes without splashing the cotton and may if necessary be

gently warmed (to about 40°). Red colour of the lower surface of the cotton indicates epihydrinaldehyde. A quantitative method using a special apparatus is also described.—K. Taüfel and Sadler, *Analyst*, 1934, 353.

Oils protected from light may remain free from rancidity for several months, but will give strong positive tests with Kreis reagent.—N. R. Col and J. A. Le Clerc, *Industr. Engng Chem., anal. Edn.*, 1934, 245.

A method based on the determination of the amount of iodine liberated from potassium or barium iodide, stated to be applicable to rancid fats of all types, even such as do not invariably give indication of rancidity with the Kreis test such as rancid cacao butter and oils blown at high temperatures, is described by A. Taffel and C. Revis, *J. Soc. chem. Ind., Lond.*, 1931, 87T.

Selective Oxidation of Animal and Vegetable Fats. Selective oxidation is used as a means to determine the purity of an animal or vegetable fat. Conditions are established under which the amount of oxidation of the more highly unsaturated acids is far greater than that of the less unsaturated and the difference between them is constant and measurable. A weighed quantity of oil or fat is treated with a solution of sodium dichromate in acetic acid for a definite time and at a definite temperature. Ferrous sulphate is then added and the excess estimated with standard potassium permanganate solution, diphenylamine oxidation-reduction indicator being used. From this an oxidation value is calculated. Fats with similar iodine value do not give similar oxidation values. The method is particularly applicable for the detection of hydrogenated fats which give low oxidation values, making their detection in other fats fairly certain.—W. A. Alexander, *Analyst*, 1938, 157.

The Bellier Values. Bellier values for different oils are as follows: arachis 38-41; coconut 10-5; cod-liver 9-5; mastic tree oil 16-75-17-75; linseed 16-5-20; olive 9-5-19; castor 0-5; soya bean 16-19; tea-seed 7-10; olive oils of different origins; Morocco 10-5; Tunis 16; Tunis with 10% arachis 19; Sfax 17; Sfax with 10% arachis oil 20-5.—R. Marcille, *Analyst*, 1940, 170.

The Blarez Value. The temperature of crystallisation of the alcoholic solution of the potassium soaps of the fatty acids obtained by saponifying 1-5 ml. oil with 15 ml. alcoholic potash (45 g. per litre). Pure olive oil 10-16, average 12; with 10% arachis oil the figure becomes 16-5; pure arachis oil 28.—R. Marcille, *Analyst*, 1940, 170.

Antioxygens Present in Oils. The naturally occurring antioxygens present in oils are destroyed when the oils are boiled with dilute mineral acids or with water. The antioxygens are present to a greater degree in the residual material of the seeds from which the oil is extracted than in the extracted oil. Concentrates have been prepared from soya-bean cake and from linseed oil and have been called inhibitor concentrates; they are probably a complex mixture of organic compounds. Considerable research has been done, see Olcott and Mattill, *J. Amer. chem. Soc.*, 1936, 1627; T. G. Green and T. P. Hilditch, *J. Soc. chem. Ind., Lond.*, 1937, 23T.

Oat starch has been shown to be of use as an antioxygen to retard the formation of rancidity in lard and oleomargarine, from 0-5 to 1-0% being added.—F. N. Peters and S. Musher, *Industr. Engng Chem., anal. Edn.*, 1937, 146.

NOTES ON INDIVIDUAL FIXED OILS

For other oils see under the corresponding drugs.

OLEUM ARACHIS

Oleum Arachis (B.P.). Sp. gr., 0-916 to 0-920. $n_{D40^{\circ}}$, 1-4625 to 1-4645; acid value, not more than 4; saponification value, 188 to 196; iodine value, 85 to 99. Complies with tests for absence of cottonseed oil, sesame oil and other vegetable oils. Huile d'Arachide, *Fr. Cx.* 1937, commences to solidify at 3°; at -5° it forms a semi-transparent fat; $n_{D25^{\circ}}$, 1-4704 to 1-4714.

The B.S.S. No. 629-1935 requires the oil to remain clear when kept at a temperature of 17-5° to 20° for 24 hours. It also includes requirements for

colour, sp. gr. (0.917 to 0.919), n_{D20}° (1.470 to 1.472), saponification value (not below 188), iodine value (85 to 99), acidity and unsaponifiable matter. The apparent proportion of arachis oil is calculated from the weight of crystals obtained in the "Evers" test after applying corrections given for solubility of arachidic acid in alcohol and a factor for converting the acid to arachis oil; it should contain not less than 96%.

To test for arachis oil in other oils, boil 1 ml. for 5 minutes under a reflux condenser with 5 ml. of 1.5 N alcoholic potash, add 1.5 ml. of acetic acid and 50 ml. of 70% alcohol, warm until clear, cool slowly to 15.5°; at this temperature it should remain clear for 5 minutes. If a precipitate is formed, 5 g. of the oil are refluxed for 5 minutes with 25 ml. of 1.5 N alcoholic potash and then 7.5 ml. of acetic acid and 100 ml. of 70% alcohol, containing 1% hydrochloric acid, added. Keep at 12° to 14° for one hour and filter, washing with the alcohol hydrochloric acid mixture, at 17° to 19°. Dissolve the precipitate in hot 90% alcohol and cool to 15° for 1 to 3 hours. Wash any crystals and dissolve in ether, evaporate and dry at 100°. The m.p., and the m.p. after recrystallisation, should be below 71°. If no crystals are formed dilute the alcohol to 70% and keep at 17° to 19° for one hour.

Arachis Oil in Other Oils. The following test for absence of arachis oil in other oils is recommended by the Sub-Committee on Fixed Oils, etc., of the Committee on Pharmaceutical Chemistry:—Boil 1 ml. of the oil in a small flask under a reflux condenser with 5 ml. of 1.5 N alcoholic potassium hydroxide for ten minutes; add 50 ml. of alcohol (70%) and 0.8 ml. of hydrochloric acid. Cool, with a thermometer in the liquid, with continuous stirring, so that the temperature falls by about 1° per minute. Note the temperature at which a turbidity appears. No turbidity appears above the following temperatures for the respective oils:—Almond oil 4°, olive oil 9°. If a turbidity is formed above the required temperature, carry out the following test:—Boil 5 g. of the oil in a 200-ml. conical flask with 25 ml. of 1.5 N alcoholic potassium hydroxide for five minutes under a reflux condenser. To the hot solution add 7.5 ml. of acetic acid and 100 ml. of alcohol (70%), containing 1 ml. of hydrochloric acid. Maintain the temperature for an hour at 12° to 14°. Filter, and wash with the same mixture of alcohol (70%) and hydrochloric acid at 17° to 19°, the precipitate being broken up occasionally by means of a platinum wire bent into a loop. The washing is continued, until the washings give no turbidity with water. Dissolve the precipitate according to its bulk in 25 to 70 ml. of hot alcohol (90%), cool, and allow it to stand at 15° for three hours. If no crystals appear arachis oil is absent. If any crystals appear, filter, and wash at 15° with about half the volume of alcohol (90%), used for crystallisation, and, finally, with 50 ml. of alcohol (70%). Dissolve the crystals in warm ether, and dry at 100°. The melting-point is lower than 71°. Re-crystallise from a small quantity of alcohol (90%); the melting-point, after drying at 100°, remains lower than 71° —(British Pharmacopœia Commission Report, No. 11, May 1939.)

OLEUM COCOIS

Oleum Cocois (B.P.C.). Solidifying-point, 22° to 23.5°; n_{D40}° , 1.4485 to 1.4495; acid value, not more than 6; saponification value, 255 to 258; iodine value, 7.9 to 9.5.

Coconut oil from copra from four sources (Inner South Sea Islands, Borneo, Celebes and Rabaul) were found not to differ significantly in their properties. The mixed fatty acids prepared by the Twitchell process, fractionated, and then the methylated fractions refractionated, were found to be composed of the saturated acids, caproic 0.3%; caprylic 9.17%; capric 9.67%; lauric, 44.05%; myristic 15.86%; palmitic 9.58%; stearic 3.16%; with a small proportion of arachidic acid and the unsaturated acids oleic 6.28% and linolic 1.53% —H. Nobori and M. Kawabata, *Analyst*, 1941 299.

A British Standard Specification (B.S.S. No. 628—1935) has been issued by the British Standards Institution for coconut oil and includes requirements regarding moisture content, colour, refractive index (1.4485 to 1.4492), iodine value (7.0 to 9.5), saponification value (not below 255), acidity (not more than the equivalent of 5.0% of lauric acid) and unsaponifiable matter (not exceeding 0.8%). The methods used for the tests and methods recommended for sampling fats and fatty oils are included in this and similar specifications.

Palm Kernel Oil. A British Standard Specification, B.S.S. No. 652—1936, has been issued for crude palm kernel oil and includes requirements regarding colour, refractive index (1.443 to 1.451 at 40°), iodine value (14 to 19), saponification value (242 to 252) and acidity (not more than the equivalent of 6% of lauric acid).

OLEUM GOSSYPII SEMINIS

Oleum Gossypii Seminis (B.P.). Sp. gr., 0.920 to 0.925; n_{D40} , 1.4645 to 1.4655; acid value, not more than 0.5; saponification value, 190 to 198; iodine value, 103 to 115. Particles of fat separate below 12° and it congeals between 0° and -5°. Complies with tests for absence of alkali, sesame oil and arachis oil.

Cottonseed oil may be detected in other oils by mixing in a tube 2.5 ml. of the oil with 1.25 ml. of amyl alcohol and 1.25 ml. of 1% precipitated sulphur in carbon disulphide and placing in a boiling water-bath; no reddish colour should develop within 30 minutes (Halphen's test). **Oleum Gossypii Seminis, U.S.P. XII**, has at 25° a sp. gr. of 0.915 to 0.921; saponification value, 190 to 198; iodine value, 105 to 114; solidification point of the fatty acids, 28° to 35°.

The B.S.S. No. 655—1936 for refined cottonseed oil does not apply to medicinal oil included in the B.P. The specification requires the oil to be the product of alkali refining the oil obtained by expression or extraction; it includes requirements regarding colour for deodorised oil and for common edible oil, sp. gr., refractive index, iodine value, saponification value (not below 192), acidity (not more than the equivalent of 0.2% of oleic acid) and unsaponifiable matter.

Oleum Lini (B.P.). Sp. gr. 0.930 to 0.940; n_{D40} , 1.4725 to 1.4750; acid value, not more than 5.0; saponification value, 187 to 195; unsaponifiable matter, not more than 1.5%; iodine value, 170 to 200. The **U.S.P. XII** oil has a sp. gr. of 0.925 to 0.935 at 25°; iodine value, not less than 170.

Raw Linseed Oil for General Purposes. The British Standards Specification for oil for general purposes, B.S.S. No. 632—1935, includes requirements for colour, sp. gr. (0.931 to 0.936), refractive index at 20° (1.4800 to 1.4835), iodine value (not below 175), saponification value (not below 188), acidity (not more than the equivalent of 2.0% of oleic acid), drying time, and unsaponifiable matter.

Linseed Oils for Paints.

The following British Standard Specifications have been prepared for linseed oil for paints:—

B.S.S. No. 242—1936 covers refined oil; the specification includes requirements regarding description, colour (not darker than fresh 0.02% iodine in 0.2% potassium iodide solution), sp. gr. (0.930 to 0.934 at 15.5°), refractive index (1.4808 to 1.4824), iodine value, saponification value, acidity, drying time, unsaponifiable matter and sampling.

B.S.S. No. 243—1936 relates to raw oil and includes requirements regarding description, colour, sp. gr. (0.931 to 0.936), n_D (1.4800 to 1.4835), iodine value, saponification value, acidity, drying time, unsaponifiable matter and sampling.

B.S.S. No. 259—1936 refers to boiled linseed oil, the specification including requirements for description, colour, sp. gr. (0.935 to 0.955 at 15° and within ± 0.002 of that of an agreed sample), acidity (not exceeding the equivalent of 4.0% of oleic acid), drying time (not more than 24 hours at 60° to 70°F.), ash (not more than 0.5%), unsaponifiable matter (not more than 2.5%), rosinate driers and sampling.

The appendices to the three specifications describe the methods to be used for the determinations of iodine value (Wijs), saponification value, acidity, drying time and unsaponifiable matter (this method is the process adopted by the Council of the Society of Public Analysts).—*Analyst*, 1933, 203.

OLEUM HIPPOGLOSSI

Oleum Hippoglossi (*B.P. Add. II*). Determined by the assay for vitamin A in relation to the Standard Preparation, contains in 1 g. not less than 30,000 units of vitamin A. The vitamin D activity varies and is usually between 2500 and 3500 units per g.; when a statement is made of the number of units of vitamin D in halibut-liver oil the units enumerated should be those described under the biological assay of vitamin D. Sp. gr., 0.924 to 0.929; acid value not greater than 1.0; saponification value not greater than 175; iodine value, not less than 115; iodine value of glycerides, 115 to 130; unsaponifiable matter, not less than 8%. Ultra-violet absorption at 300 m μ not more than 75% of that at 328 m μ . *B.P. Add. IV* alters the acid value to not more than 6.0, the iodine value to not less than 112, the iodine value of glycerides to 112 to 130, and the unsaponifiable matter to not less than 7%. Oleum Hippoglossi, *U.S.P. XII*, contains not less than 60,000 *U.S.P.* units of vitamin A and not less than 6000 *U.S.P.* units of vitamin D per g., and may be flavoured by the addition of not more than 1% of an official flavouring substance. Sp. gr. at 25°, 0.920 to 0.930; unsaponifiable matter, 7 to 13.5%; saponification value, 160 to 180; iodine value 125 to 155. 2 g. contains free fatty acids equivalent to not more than 1 ml. of N/10 sodium hydroxide.

Determination of the Iodine Value of the Glycerides of Halibut-liver Oil (*B.P. Add. II*). Determine the iodine value of the oil by the pyridine bromide method (x). Determine the unsaponifiable matter (S) using 1 g. of the oil, evaporating the acetone from the unsaponifiable matter by means of a current of nitrogen and drying the residue at 80° in a current of nitrogen. Weigh the residue and immediately determine the iodine value by the pyridine bromide method (y). Calculate the iodine value of the glycerides from the formula:

$$\frac{100x - Sy}{100 - S}$$

Determination of Ultra-violet Absorption. The *B.P. Add. II* directs that the ultra-violet absorption of halibut-liver oil is determined at 328 m μ and at 300 m μ on a solution of the oil in cyclohexane.

Blue values and iodine values of samples of halibut-liver oils of known origin compared with those of samples of the commercial oil and mixtures with cod-liver oil.—R. T. M. Haines and J. C. Drummond, *J. Soc. chem. Ind., Lond.*, 1934, 81T.

The "blue values" of samples of halibut-liver oil examined varied from 300 to 1720.—R. T. M. Haines and J. C. Drummond, *Brit. med. J.*, i/1933, 558.

Vitamin A Content of Fish-liver Oils (other than Cod). The range of vitamin A concentration is at least 2500 : 1 and is not parallel with vitamin D potency. The liver oils of haddock, whiting, skate of medium or small size, codling, and immature or small fish generally, are poor in vitamin A. Oils from

pollack, saithe, hake and ling are similar in potency to cod-liver oil; salmon, turbot, sturgeon and especially halibut yield liver oils rich in vitamin A. The larger halibut occurring in northern waters yield the better liver oil. Unlike the oils of other fish, halibut-liver oil shows a seasonal variation in vitamin A content independent of the spawning period. The oil is richest in vitamin A in May, subsequently falling, and rising to a second smaller maximum in September. There is possibly a connection between vitamin A content and the abundance of planktonic organisms from which the fish probably obtain carotene for conversion to the vitamin.—J. A. Lovern, J. R. Edisbury and R. A. Morton, *Biochem. J.*, 1933, 1461.

Oils obtained from the stomach, mesentery and intestine contain up to ten times as much vitamin A as does halibut-liver oil, and as large a quantity may be obtained from the viscera as from the liver although the latter is of considerably greater weight.—J. A. Lovern, *Nature, Lond.*, ii/1937, 276.

Analytical Data

It is the practice to standardise oils for sale to a definite vitamin A content. This may be done either by mixing strong and weak halibut-liver oils to give the desired vitamin content or by diluting a strong halibut-liver oil with another fish-liver oil, such as cod-liver oil, or even with a vegetable oil. There seems no objection to this practice, but if any other oil is added the product cannot be described as natural halibut-liver oil. The following variations were found in 33 samples of halibut-liver oils obtained during two seasons: Blue value, 205 to 7100; vitamin A $E_{1\text{cm}}^{1\%}$, 328 m μ , 6.8 to 144; sp. gr., 0.922 to 0.9286; n_{D40° , 1.470 to 1.488; iodine value, 111 to 171; saponification value, 150 to 175; unsaponifiable matter, 8.3 to 21.5%.—N. Evers and W. Smith, *Pharm. J.*, i/1935, 417.

Results are recorded of analyses of 30 samples of halibut-liver oil. The following ranges were found:—Blue value, 569 to 12,930; iodine value, 114 to 161; n_{D40° , 1.4688 to 1.4887; unsaponifiable matter, 6.34 to 17.4; sterols in unsaponifiable matter, 31.1 to 73.8. There is an inverse relationship between the amounts of sterol and of vitamin A.—R. T. M. Haines and J. C. Drummond, *Analyst*, 1936, 2.

The following ranges were obtained in analyses of 46 samples of halibut-liver oil during the 1935 season; average values obtained are included in brackets—Vitamin A, Blue value 495 to 6300 (1810); units per g. ($E_{1\text{cm}}^{1\%} \times 1600$) 18,700 to 211,300 (58,620). Vitamin D (determined on 3 bulked samples of the 46 batches), 2300 to 2800 (2560) units per g. Sp. gr. 0.924 to 0.929 (0.9265); n_{D40° , 1.4709 to 1.4836 (1.4739). Saponification value, 160 to 176 (171.2). Iodine value, 115 to 131 (121.4). Unsaponifiable matter % 7.2 to 17.55 (10.42). Figures are also given for the acid values and for the iodine values of the unsaponifiable matter, the glycerides and the non-vitamin A unsaponifiable matter.—N. Evers, A. G. Jones and W. Smith, *Analyst*, 1936, 7.

OLEUM HYDNOCARPI

Oleum Hydnocharpi (B.P.). Sp. gr. (25°/25°), 0.950 to 0.960; m.p., 20° to 25°; specific rotation of a 10% w/v solution in chloroform, not less than +53°; n_{D40° , 1.472 to 1.476. Acid value, not more than 25; saponification value, 198 to 204; iodine value 97 to 103.

Oleum Hydnocharpi Æthylicum (B.P.). Sp. gr., 0.905 to 0.910; α_D , not less than +45°; n_{D20° , 1.458 to 1.462; acid value, not more than 1.0; saponification value, 190 to 196; iodine value, 88 to 94. Æthyliis Chaulmoogras, *U.S.P. XII*, has a sp. gr. of about 0.904 at 25°; specific rotation, on a 50% v/v solution in chloroform, not less than +44.5°; saponification value, 190 to 196; iodine value, 90 to 100.

Oleum Chaulmoogræ (B.P.C.). M.p. about 25°. Sp. gr., about 0.95 at 25°; specific rotation at 20° on a 10% w/v solution

in chloroform, +48° to +52°; acid value, 22 to 30; saponification value, 196 to 213; iodine value, 98 to 104. M.p. of the mixed fatty acids, 44° to 45°. Oleum Chaulmoogræ, *U.S.P. XII*, complies with a limit for free fatty acids, a test for free fatty acids or castor oil, has a saponification value of 196 to 213, and an iodine value of 93 to 104.

Chemical Composition of the oil from the seeds of *Hydnocarpus Kurzii*. By distillation of the ethyl esters *in vacuo* at below 0.05 mm., by which means the last 10 to 15% of the esters were obtained without appreciable decomposition, the following substances were isolated: taraktogenic acid, $C_{38}H_{76}O_8$, m.p. 113.5°, iodine value 42.51; isogadoleic acid, $C_{20}H_{38}O_2$, m.p. 65.5° to 66°; a lactone-like substance, $C_{16}H_{32}O_2$, m.p. -11.6°; an acid, possibly arachidic acid; a brown resinous material insoluble in organic solvents; two unidentified solids probably acids. The presence of chaulmoogric and hydnocarpic acids, and these only, in the lighter fractions of the esters of the non-volatile acids is confirmed.—T. Hashimoto, *J. Amer. chem. Soc.*, 1925, 2325.

The characteristics of more than half the samples of commercial chaulmoogra oils examined approximate closer to those of *H. anthelmintica* or *H. Wightiana* than to those of *H. Kurzii*.—E. Andre, *C. R. Acad. Sci., Paris*, ii/1925, 1081, per *Quart. J. Pharm.*, 1926, 133.

Oil of *Hydnocarpus ilicifolia* (*Taraktogenos subintegra*) has been prepared from seeds grown in Siam; it has the following values: m.p., 23° to 28.2°, acid value as oleic acid 0.6%, sp. gr. 30°/4°, 0.947, saponification value 213.1, iodine value 89.7, n_D^{20} , 1.4763.—A. Marcon, *J. Soc. chem. Ind., Lond.*, 1926, 305T.

The oil employed in the treatment of leprosy is obtained from seeds of various species of *Hydnocarpus*. The chief amongst these are *H. Wightiana* Blume, *H. anthelmintica* Pierre and *H. Kurzii* Warb (= *Taraktogenos Kurzii* King). The last was formerly the chief source of the oil used in India, whilst *H. anthelmintica* of Indo-China and Siam was used in China. It has been found that the most effective oil is that of *H. Wightiana*, an Indian species, and this oil is the only one recognised in the B.P. Oil obtained from the seeds of *H. Wightiana* from Nigeria complied with the B.P. tests for purity but, possibly owing to its low free acid content, was not soluble in hot 90% alcohol. Oil from seeds grown in Ceylon also complied with B.P. requirements whilst oil from Malaya seeds had a saponification value of 205.1, but otherwise conformed.—*Bull. imp. Inst., Lond.*, 1936, 145.

Other oils of the Chaulmoogra group compared with the oil of *Hydnocarpus Kurzii* are:—

| Source | Sp. gr. | n_D | α_D | m.p. | Sap. value | Iodine value |
|---|------------|------------|------------|----------------|------------|--------------|
| <i>Hydnocarpus Kurzii</i> .. | 0.9425/32° | — | +48°00' | 33° to 39° | 210.4 | 96.1 |
| <i>Hydnocarpus anthelmintica</i> (Krabao Oil) | 0.9447/29° | 1.4755/29° | +58°10' | 26° to 29° | 191.0 | 90.0 |
| <i>Hydnocarpus anthelmintica</i> | 0.9427/32° | 1.4742/29° | +48°00' | 25° to 26° | 187.3 | 88.3 |
| <i>Hydnocarpus Wightiana</i> (Kavatel Oil) | 0.9330/32° | 1.4780/29° | +61°40' | 28° to 32° | 197.2 | 103.0 |
| <i>Hydnocarpus alpina</i> .. | 0.9346/32° | 1.4764/29° | +57°00' | 20.5° | 201.0 | 95.0 |
| <i>Asteristigma macrocarpa</i> .. | 0.9217/32° | 1.4725/25° | +44°00' | 37° to 39° | 180.4 | 82.8 |
| <i>Oncoba echinata</i> (Gorli fat) .. | 0.9286/32° | 1.4740/31° | +56°10' | 40.4° to 41.5° | 184.5 | 98.0 |
| <i>Carpotroche brasiliensis</i> .. | 0.9499/32° | 1.4755/31° | +53°40' | 21° to 23° | 183.7 | 106.1 |

Percentage Composition of the Fatty Acids of Chaulmoogra Oils.

| | <i>H. wightiana</i> | <i>H. anthelmintica</i> | <i>Taraktogenos kurzii</i> |
|--|---------------------|-------------------------|----------------------------|
| Hydnocarpic | 48.7 | 67.8 | 34.9 |
| Chaulmoogric | 27.0 | 8.7 | 22.5 |
| Gorlic | 12.2 | 1.4 | 22.6 |
| Oleic | 6.5 | 12.3 | 14.6 |
| Palmitic | 1.8 | 7.5 | 4.0 |
| Lower homologues of chaulmoogric acid .. | 3.4 | 0.1 | 0.4 |
| Loss | 0.4 | 2.2 | 1.0 |

—C. H. Cole and H. T. Cardoso, *Int. J. Leprosy*, 1941, 215.

Thiocyanate and Iodine Values. Fatty acids or glycerides with one double bond react towards thiocyanogen in the same way as towards iodine, but if they contain two double bonds, only one reacts with thiocyanogen, though both react with iodine. Thus the thiocyanogen value (expressed as iodine) of linolic acid is only half the iodine value. These values for chaulmoogra and hydnocarpus oils were:—

| | | |
|--------------------|--------------------|------------------------|
| Chaulmoogra Oil .. | Iodine value 100.6 | Thiocyanate value 99.1 |
| Hydnocarpus Oil .. | " " 95.7 | " " 94.8 |

indicating that little or no acids of the linolic series were present.—E. I. van Itallie, *Quart. J. Pharm.*, 1929, 618.

Ethyl Esters. The esterified oil of *Hydnocarpus Wightiana* when fractionated yields almost pure ethyl hydnocarpate; if chaulmoogra oil is used there is contamination with ethyl palmitate.—C. A. Perkins, A. O. Crusy and M. O. Riges, *Industr. Engng Chem., anal. Edn.*, 1927, 939.

Tests for Suitability of Hydnocarpus Oil for Injection. Various specimens of the oil have been examined physically and chemically in the Leprosy Department of the School of Tropical Medicine, Calcutta, and the findings correlated with the results of therapeutic tests on patients. Pain and other undesirable reactions following injection of the oil were found to be due to the use of oil which had been badly prepared, badly stored, or both. The oil should be expressed from sound, fresh, ripe seeds only. If the outer part of the seeds is not removed before expression the oil should be filtered as soon as possible since the presence of dirt and dust, as also exposure to air, light and moisture, favours oxidation and increase in acidity. Repeated sterilisation also aids oxidation and the oil should be sterilised once only. The acidity of a good oil, expressed as oleic acid, is not more than 1%, but an acidity of 3% is usually satisfactory; 3 to 5% of acid indicates a doubtful oil while with more than 5% the oil is usually poor. A test for peroxide gives a more reliable indication of the irritant properties of an oil than the determination of acidity. The following qualitative test is suggested: To 1 ml. of oil add 1 g. of solid potassium iodide and about 5 ml. of a mixture of glacial acetic acid and chloroform (2 : 1), shake and boil vigorously for 30 seconds. In the presence of a marked excess of peroxide a brown colour develops. If there is no appreciable colour, cool and add 10 ml. of 5% potassium iodide solution and 1 ml. of starch solution. The oil is not suitable for injection if the colour is deeper than light brown. The test can be modified so as to yield quantitative results by means of a titration with thiosulphate. The test for peroxides cannot be applied to solvent extracted oils or to oils which have been washed with sodium hydroxide solution.—J. Lowe and N. K. De, *per Leprosy Rev.*, 1938, 32.

OLEUM MORRHUÆ

Oleum Morrhuae (B.P. Add. I). Determined by the assay for vitamin A in relation to the Standard Preparation, contains in 1 g. not less than 600 units of vitamin A, and determined by the

biological assay of antirachitic vitamin (vitamin D) in relation to the Standard Preparation, contains in 1 g. not less than 85 units of antirachitic activity (vitamin D). Sp. gr., 0.922 to 0.929; n_{D40}^{20} , 1.4705 to 1.4745; acid value, not greater than 1.2; saponification value, 180 to 190; unsaponifiable matter, not more than 1.5%; iodine value, 155 to 173. Remains bright when kept at 0° for 3 hours. Oleum Morrhuae, U.S.P. XII, contains not less than 850 U.S.P. Units of vitamin A and 85 U.S.P. Units of vitamin D in each gramme. Complies with a test for stearin and a colour test when matched against a standard solution of cobaltous chloride and ferric chloride. It has a sp. gr. of 0.918 to 0.927 at 25° and an iodine value of 145 to 180; unsaponifiable matter, not more than 1.3%. The Fr. Cx. 1937 requires the oil to contain 600 International Units of vitamin A and 85 International Units of vitamin D, determined biologically. Vitamin A may be determined spectroscopically.

The percentage of unsaponifiable matter is a guide to purity, as oils likely to be used as adulterants mostly have high unsaponifiable matter. The lower limit for genuine cod-liver oil was 0.56% and the upper limit 1.5%, with an average of 1.07% determined on 150 samples representing 30,000 tons of oil.—C. C. Harris, *J. Soc. chem. Ind., Lond.*, 1938, 508.

Arsenic Content of twenty samples of crude American cod-liver oil from various producing centres was found to vary from 1.4 to 5.1 parts per million.—R. Remington, *Industr. Engng Chem., anal. Edn.*, 1934, 573.

Colour Standards. As the result of the examination of fifty-four samples two permanent colour standards, for light and dark oils, are suggested. For light oils, the oil when placed in a four-ounce cylindrical standard oil-sample bottle and viewed transversely shall not be more highly coloured than a solution placed in a similar bottle, containing 3.6 ml. of M/4 $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 48.4 ml. of M/6 $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 68 ml. of distilled water. For dark oils the solution is, 11 ml. of M/4 $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 76 ml. M/6 $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, and 33 ml. of distilled water. The M/4 $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ solution contains 59.4965 g. of salt in 1000 ml. of 1% hydrochloric acid, the M/6 $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution contains 45.054 g. of salt in 1000 ml. of 1% hydrochloric acid.—A. Traub, *J. Amer. pharm. Ass.*, 1933, 194.

Nearly all samples of American cod-liver oil complied with the above standards but about one-third of the European oils were unsatisfactory as regards colour. Old oils which had been stored under unsatisfactory conditions were nearly all as pale as fresh oils, the acid values were however higher. As a rule dark oils have higher acid values than pale oils but there is no correlation between colour and acid value.—F. Tripp, *J. Amer. pharm. Ass.*, 1933, 1102.

Determination of Ultra-violet Absorption. The B.P. Add. II directs that the ultra-violet absorption is determined at 328 μ on a solution of the unsaponifiable matter in dehydrated alcohol or cyclohexane.

Viscosity of Cod-liver Oils. The viscosity is much influenced by temperature, so that appearance will not give an approximate estimation. When determined by the U.S.P. IX method for liquid paraffin the figures given for cod-liver oil at 25° are very uniform, nine brands of oil giving figures varying from 5.39 to 5.8 and ten successive batches from a single source 5.55 to 5.8.

Determination of Cod-liver Oil in Emulsions. To 10 g. of the emulsion is added 2.5 ml. of dilute sulphuric acid and the mixture shaken out twice with 20 and 20 ml. of chloroform, each time for two minutes. The chloroform is run into a small flask, dried with 1 or 2 g. of anhydrous sodium sulphate and filtered into a tared dish. The sodium sulphate and filter are washed twice with 5 ml. of chloroform. A current of dry air is drawn through the flask which is immersed in a water-bath. After the removal of the chloroform the residue is weighed.—R. Brieger, *Yearb. Pharm.*, 1913, 100.

To 10 g. of the emulsion is added 25 ml. of water and 5 ml. of alcohol. After gently warming, the mixture is shaken out with 30, 30 and 30 ml. of ethy ether.

The bulked ether extract is dried with anhydrous sodium sulphate, the solvent distilled from a tared flask, and the residue dried for 15 minutes and weighed.—C. Mannich and L. Schwedes, *Yearb. Pharm.*, 1913, 101.

A method using a modified cassia or Hirschon flask is described in *Yearb. Pharm.*, 1914, 235. Into an Erlenmeyer flask weigh 1 to 2 g. of emulsion, add 10 ml. of 12.5% hydrochloric acid and a small piece of pumice, heat gently over a wire gauze until a clear solution results, partly closing the flask with a small funnel. Pour the warm liquid into a 100 ml. cylinder (graduated to 0.5 ml.) and having an internal diameter of 2 cm., wash out the flask, after cooling, with 30 ml. each of ether and petroleum ether, adding these solvents to the contents of the cylinder and shaking vigorously. After standing 1 to 2 hours, note volume of ethereal solution, withdraw 50 ml. to a tared 150 ml. round-bottom flask, washing out the pipette with petroleum ether. Distil off the ether and petroleum ether, dry at 100° and weigh.—G. Buemning, *Yearb. Pharm.*, 1915, 262.

10 g. of the diluted emulsion (1 in 10) is mixed with 2 ml. of solution of ammonia, 10 ml. of dehydrated alcohol, 20 ml. of ethyl ether and 20 ml. of petroleum ether, well shaking after the addition of each. Allow to stand for 15 minutes, draw off the aqueous liquor and shake the ethereal layer with 0.6 g. of tragacanth. After clearing, the ethereal liquid is filtered into a tared beaker, the tragacanth washed with two portions, each of 5 ml., of petroleum ether which is also filtered into the beaker. The combined ethereal liquors are evaporated, the residue dried at 100° to constant weight.—Scriba, *Yearb. Pharm.*, 1915, 262.

The determination, if carried out in a Soxhlet, is unsatisfactory because of the oxidation which occurs when the emulsion is mixed with sand and dried. Satisfactory results may be obtained by a modification of the Rose-Gottlieb method. 10 g. of the emulsion is heated for ten minutes with 10 g. of 25% hydrochloric acid, cooled, transferred to a separator, rinsed in with 10 ml. of alcohol followed by 3 portions each of 10 ml. of a mixture of equal parts of ether and petroleum ether. 50 ml. of water is added and the bottle shaken vigorously. After separation, run off the aqueous liquid and add 1 g. of tragacanth. The oil solution is dried at 100° and weighed.—Valentin, *Quart. J. Pharm.*, 1932, 78.

Determination of Cod-liver Oil in Malt and Oil. The method is based on the fact that the sp. gr. of cod-liver oil is less than that of water and that of malt extract is considerably greater, and further that the sp. gr. of a 50% solution of malt extract is, within small limits of error, proportional to the n_D of the malt extract itself. Equal weighed quantities of the sample and water are mixed and the sp. gr. determined at 20°. The sp. gr. may be corrected by adding 0.0006 for each 1° above if taken at over 20°. The n_D is determined at 20°, correcting by the addition of 0.00018 for each 1° if taken at over 20°. The percentage of oil is calculated from the formula:—

$$(n_D - 1.3332) \times 549.6 - \frac{(d-1)}{d} \times 584.8.$$

—H. D. Richmond and F. G. Hitchman, *Yearb. Pharm.*, 1917, 75.

Use 4 to 6 g. of the sample, add 15 ml. of water, mix, add 50 ml. of ethyl alcohol, place in a 250 ml. separating funnel and shake until the emulsion breaks, add 50 ml. of low boiling petroleum ether and shake for a few minutes, separate the hydro-alcoholic solution and the petroleum ether solution into two beakers. Replace the hydro-alcoholic solution in the separator and repeat the extraction with petroleum ether four or five times. Evaporate the combined petroleum ether extracts. Dry the residue in a desiccator over sulphuric acid to constant weight.—C. S. Waggoner and C. C. Glover, *J. Amer. pharm. Ass.*, 1926, 754.

Gunn-Venables Method. In order to overcome the formation of emulsions when the diluted malt preparation is shaken with ether and to avoid loss of vitamin A from the oil, weigh accurately 5 to 10 g. into a beaker and mix with distilled water (3 ml. for each gramme taken). Add 8 parts of dehydrated alcohol to each 5 parts of the above dilution (industrial methylated spirit may be used) and 0.4 g. of purified kaolin. Stir thoroughly and allow the precipitate to settle. Filter the clear liquid and add 50 ml. of 70% alcohol to the precipitate, again stir and allow to settle. Filter the clear liquid through ordinary grade paper, and collect the residue on the filter, washing out the

beaker with 70% alcohol. When the precipitate has drained release any water from the stem of the funnel, and wash the residue with ether, collecting the ether in a separating funnel. Any precipitate adhering to the paper should be detached with a glass rod. Continue washing with ether until a drop shows no film on evaporation, about 160 ml. being necessary. Wash the ether in the separator with three portions of 30 ml. of distilled water and transfer to a distilling flask. Distil off the ether and transfer the residue to a weighed beaker, washing out the flask with a little ether. Add a few ml. of dehydrated alcohol, evaporate, cool and weigh. The oil may then be tested by the methods of the *B.P.C.*—Gunn and P. F. R. Venables, *Quart. J. Pharm.*, 1936, 430.

Rose-Gottlieb Method. Of various methods considered, the Rose-Gottlieb and Gunn-Venables methods were found to be most suitable. With fresh materials both methods gave results that agreed well and afforded an accurate measure of the oil-content, but with increase in age of the preparation the latter method gave low results. The vitamin A content of the extracted oil was determined spectrophotometrically and it was shown that full recovery could be obtained when the Rose-Gottlieb method of extraction of the oil was used, provided that precautions were taken against oxidation. The details of the method are as follows. Disperse about 5 g. of the preparation in 5 ml. of water and transfer the mixture to a stoppered 100-ml. tube by means of a further 5 ml. of water. Add 1 ml. of ammonia solution (sp. gr., 0.880) and 5 ml. of alcohol followed by 20 ml. of ether, using part to rinse in any oil possibly remaining in the original weighing vessel. Stopper and shake vigorously for two or three minutes. Add 20 ml. of light petroleum (40°–60°) and shake again. Centrifuge, siphon off the solvent layer into a tared flask and repeat the shaking and extraction with about 15-ml. portions of ether and light petroleum. Evaporate, re-evaporate after addition of a few ml. of alcohol or acetone, dry and weigh the oil. Precautions must be taken against oxidation such as evaporating in an atmosphere of nitrogen and keeping ethereal solutions under nitrogen. In determining the vitamin A content, the oil was not dried, but immediately saponified with alcoholic potash for a maximum of five minutes. The unsaponifiable matter was extracted by the method given in the *B.P. Add. I*, and the solution obtained evaporated under nitrogen. A few ml. of pure acetone were added, the liquid re-evaporated and the unsaponifiable matter cooled in nitrogen. It was dissolved in spectroscopically pure cyclohexane, diluted to a suitable volume and immediately examined spectrographically.—D. C. Garratt, *Analyst*, 1939, 795.

The following details for the Rose-Gottlieb method for the determination of oil are recommended by the Sub-Committee on the Assay of Galenicals of the Committee on Pharmaceutical Chemistry:—Mix about 2.5 g., accurately weighed, with 5 ml. of water and transfer to a 100-ml. stoppered cylinder, using a further 5 ml. of water. Add 5 ml. of alcohol (95%), 1 ml. of strong solution of ammonia and 25 ml. of ether. Shake vigorously for a few minutes; add 25 ml. of light petroleum (boiling-point, 50° to 60°); shake and allow to stand until the ethereal layer is quite clear. Siphon off the ethereal layer; add 20 ml. of ether to the residual aqueous layer and shake vigorously; add 20 ml. of light petroleum (boiling-point, 50° to 60°). Shake and allow to stand until the ethereal layer is clear. Siphon off the ethereal layer and repeat the extraction, using a further 20 ml. of ether and adding 20 ml. of light petroleum (boiling-point, 50° to 60°). Mix the ethereal solutions and remove the solvent by evaporation on a water-bath in an atmosphere of nitrogen; dry the residue in an atmosphere of nitrogen at 100° and weigh.—(*British Pharmacopœia Commission Report*, No. 11, May 1939.)

Werner-Schmidt Method. In the determination of oil in extract of malt with cod-liver oil the low results obtained by the Gunn and Venables method are due to the almost quantitative solubility of the fatty acids in the alcohol used, the figure obtained for extracted oil being that of neutral oil only. It was found that the acidity of the oil in extract of malt with cod-liver oil increases, in some cases, to an extraordinary degree. In thirty samples examined the free acidity of the extracted oil calculated as clupanodonic acid varied from less than 2.5% in six samples to above 10% in seven samples. For the determination of the total fatty matter the Werner-Schmidt method modified as follows was used:—Disperse about 2.5 g. of extract of malt with cod-liver oil in 15 ml. of warm water, add 15 ml. of concentrated hydrochloric acid, transfer to a 200 ml. separator, washing in with 10 ml. of water and 50 ml. of ether. Shake and

separate; re-extract the aqueous layer with 30 ml. of ether. Wash the combined ether extracts free from mineral acid, re-extract the washings with ether, bulk the ethers, evaporate, re-evaporate twice with a little acetone and dry in a desiccator for half-an-hour before weighing.

The extracted oil is titrated for free acidity in the usual way. The method is not suitable for routine analysis as persistent emulsions are liable to be formed which are eliminated with difficulty. A limit of 5% acidity in the oil is suggested for the *B.P.* preparation.—D. C. Garratt and J. E. Woodhead, *Quart. J. Pharm.*, 1941, 14, 172–175.

A tentative method for the determination of cod-liver oil in emulsions. The sample is mixed with calcium carbonate and extracted with chloroform. The chloroformic extract is evaporated and the oil weighed.—*Methods of Analysis (A.O.A.C., 1940, 625).*

THE VITAMINS OF COD-LIVER OIL

Cod-liver oil contains vitamins A and D in varying amounts. Most samples of cod-liver oil have a vitamin D potency between 60 and 150 international units per gramme; occasionally samples are found to have 300 or 350 units per gramme. The vitamin A potency ranges from about 1000 to 3500 international units per gramme, though occasionally samples are found to have a lower or a higher value than these.

There is no correlation between the vitamin A and the vitamin D values of a sample of cod-liver oil. The following table of values found for 23 samples of cod-liver oil in the Pharmaceutical Society's Laboratories shows this:—

Vitamin A and D Value of Twenty-three Samples of Cod-Liver Oil Examined Biologically

| Sample | Vitamin A International Units | Vitamin D International Units |
|--------|----------------------------------|----------------------------------|
| 1 | 1,500 | 160 |
| 2 | 1,500 | 360 |
| 3 | 500 | 80 |
| 4 | 650 | 85 |
| 5 | 1,500 | 210 |
| 6 | 3,600 | 250 |
| 7 | 2,000 | 120 |
| 8 | 8,400 | 150 |
| 9 | 4,500 | 80 |
| 10 | 1,500 | 80 |
| 11 | 3,000 | 300 |
| 12 | 3,700 | 300 |
| 13 | 1,500 | 330 |
| 14 | 4,800 | 300 |
| 15 | 1,500 | 340 |
| 16 | 2,250 | 240 |
| 17 | 1,250 | 220 |
| 18 | 2,750 | 60 |
| 19 | 1,500 | 290 |
| 20 | 2,000 | 50 |
| 21 | 1,500 | 110 |
| 22 | 2,850 | 250 |
| 23 | 750 | 180 |

The vitamin value of cod-liver oil seems to vary inversely with the yield of oil from any particular liver or batch of livers. The origin of the vitamins in cod-liver oil is almost certainly the diet of the cod, namely herring, whiting, codlings in the North Sea, and squid and caplin round the Newfoundland banks. Since, however, these have only a low vitamin content, it must be concluded that the cod itself uses little of the vitamin obtained from its food but stores it in the liver for some unknown reason.—Drummond and Hilditch, *Empire Marketing Board Publication, No. 35, 1930, H.M.S.O.*

The oil obtained from the liver of the soup-fin shark (caught off the Pacific Coast of the U.S.A.) has a higher vitamin A potency than that of almost any other fish, amounting, for example, to more than 30 times that of cod. More

than 60% of the national production of vitamin A in the U.S.A. in 1940, i.e., 25 billion units, were manufactured from this source.—*Chem. & Drugg.*, ii/1942, 205.

Blue values determined by the B.P. method on 67 oils purchased from retail chemists were obtained ranging from 3.5 to 20.0 and the average was 7.8. The average blue value obtained on 36 samples via the unsaponifiable matter was 27.0. The best values for average oil are:—direct blue value=9.3; blue value via unsaponifiable matter=21.8. The best values obtained by spectrophotometric examination are:—extinction coefficient (1% solution in 1 cm. cell) at $328\text{ m}\mu=0.587$ (on oil) and 0.505 (via unsaponifiable matter); percentage of vitamin A=0.0315. The biological assays of bulked samples gave the following results:—vitamin A=670 units per gramme; vitamin D=81 units per gramme.—R. S. Morgan and H. Pritchard, *Analyst*, 1935, 357.

An Alternative Colorimetric Method. The determination of vitamin A in cod-liver oil can be made by a colour test in which the oil is heated with a chloroform solution of catechol and antimony trichloride. The violet-red colour obtained is compared as soon as it is formed with a 0.01% solution of potassium permanganate. The colour is said to be more stable than the blue colour obtained with antimony trichloride alone.—E. Rosenthal and J. Erdélyi, *Biochem. J.*, 1934, 28, 41.

Effect of Light on Vitamin A Potency. Exposure of cod-liver oil to any source of white light of sufficient intensity results in "delumination"—disappearance of normal golden fluorescence—and destruction of vitamin A. Kept for some months in the dark it regains much of its fluorescence but vitamin A is permanently destroyed.—P. R. Peacock, *Lancet*, ii/1926, 329.

Cod-liver oil emulsions can be kept for at least 4 months without appreciable loss of vitamin A potency, and probably for 7 or 8 months without serious alteration, if stored in well-stoppered, amber glass bottles and kept in the dark.—H. N. Griffiths, T. P. Hilditch and J. Rae, *Analyst*, 1933, 65.

Unsaturated Fatty Acids in the Oil. Cod-liver oil is composed almost entirely of unsaturated fats. An increase in the amount of unsaturated fatty acids in the environment of the tubercle bacillus tends to disintegrate it. An increase of unsaturated fatty foods yields an increase of the same in the blood, and the bacillus is present in the blood in comparatively early cases of phthisis. Increase of saturated fat above a certain point retards its absorption from the intestine. The saturated fat is assimilated up to about 14% only; unsaturated, on the other hand to the extent of 98%. In a mixed diet the unsaturated help the saturated to become absorbed. The highly unsaturated acids serve the immediate needs of energy production and the saturated are stored in the nature of a reserve.

Cod-liver oil should be prepared under conditions preventing oxidation, both from the aspect of this theory and the vitamin content.

Cod-liver oil is a food—it enters into permanent combination with a body cell yielding energy to it and altering the whole of the cell's relations by becoming an integral part of the cell protoplasm. It is more readily absorbed than other fats and has probably a marked action on metabolism.

A part of the fat obtained from animal tissues is not real fat, but complex combinations of fatty acids with glycerophosphoric acid and nitrogen-containing compounds—the so-called phosphatides—this is the portion actually made use of by living cells. Phthical patients treated with cod-liver oil have been observed and effects on *nitrogen metabolism* found to be marked and the beneficial effect on *fat absorption* to be considerable.

The total activity of cod-liver oil does not reside in its vitamin D, or in A+D, any more than quinine represents the total activity of cinchona bark. Attention is drawn to the phosphatides and the high content of *unsaturated fatty acids*.—J. W. England, *J. Amer. pharm. Ass.*, 1929, 116.

British Standards Specifications for veterinary cod-liver oil and for whale oil have been prepared as follows:—

B.S.S. No. 839—1939, relates to veterinary cod-liver oil and includes requirements regarding description, colour, sp. gr. (0.922 to 0.929), refractive index for the D line at 20° (1.478 to 1.482), iodine value (150 to 178), acidity (not to exceed 1% unless otherwise declared, and in no case to exceed 3% expressed as oleic acid), unsaponifiable matter, vitamin potency (not less than 500 i.u. of vitamin A and 50 i.u. of vitamin D), blue value (not less than 6) and sampling.

B.S.S. No. 836—1939, relates to whale oil and includes requirements regarding description, moisture and dirt, colour, saponification value (182 to 205), acidity (not more than 6% calculated as oleic acid), unsaponifiable matter and sampling.

See also under Vitamins, p. 760

Oleum Morrhue Non-Destearinatum (U.S.P. XII). Contains not more than 0.5% *v/v* of water and liver tissue, not less than 850 *U.S.P.* Units of vitamin A and 85 *U.S.P.* Units of vitamin D in each gramme; iodine value, 128 to 180.

Sodii Morrhuas (B.P. Add. IV). Forms a clear solution in 10 parts of warm water. Iodine value of fatty acid, 140 to 175. It complies also with limit tests for alkali hydroxide or free fatty acid, alkali carbonate and oxidised fatty acids.

Comparison of five samples showed that "Sodium Morrhuate 10%" is very differently interpreted by different makers and that some standards should be laid down and conformed to. Iodine value varied from 148 to 226.5, colour from clear yellow to turbid orange, and fatty acid content from 7.0 to 9.45%. —R. T. M. Haines, *Lancet*, i/1933, 748.

Liquor Vitaminæ A (B.P.C.). Contains in 1 g. approximately 60,000 units. The unit is the activity of 0.0006 mg. of a sample of β -carotene kept in the National Institute for Medical Research, London, and adopted as the international standard.

OLEUM OLIVÆ

Oleum Olivæ (B.P.). Sp. gr., 0.915 to 0.918; n_{D40}^{20} , 1.4605 to 1.4635; acid value, not more than 2.0; saponification value, 190 to 195; iodine value, 79 to 88. Complies with tests for absence of sesame oil, cottonseed oil and arachis oil. The *B.P. Add. I* alters the test for sesame oil—equal volumes of the oil and an alcohol (90%) and strong solution of ammonia mixture (9 : 1) are shaken and the alcohol and ammonia evaporated on a boiling water-bath; the oil is then shaken with half its volume of 1% sucrose in hydrochloric acid; after 5 minutes in the acid layer there should be no pink or no deeper pink than a control test without sucrose. The *Fr. Cx.* 1937, in testing for arachis oil, separates the ether-insoluble lead soap from 20 g. of oil and dissolves the regenerated fatty acid in 100 ml. alcohol (90%) with one drop of dilute hydrochloric acid; the crystals forming in the solution on cooling have a m.p. of 70° to 75°. Sesame oil is excluded by adding to 2 or 3 drops of 2% furfural in alcohol (95%) solution 10 ml. of the oil followed by 10 ml. of hydrochloric acid—the separated acid should not be coloured red. Olive oil should be decolorised by warming to 50° with animal charcoal before testing for cottonseed oil, and the reaction tubes are immersed in boiling brine for a longer period. The *U.S.P. XII* oil has a sp. gr. of 0.910 to 0.915 at 25° and an iodine value of 79 to 88; solidification point of the fatty acids, 17° to 26°.

The *B.S.S. No. 630—1935* does not differentiate between virgin and refined olive oils. It includes requirements regarding description, colour, specific gravity, refractive index (1.468 to 1.471 at 20°), iodine value, saponification

value, acidity and unsaponifiable matter, and gives details of the tests to be applied for the absence of arachis, sesame and cottonseed oils.

Prevention of Deposit. The addition of 0.1 to 0.5% of air-blown cacao butter to olive oil enables the oil to be stored for long periods at 2° without any deposition of stearin. The addition has no effect on arachis or cottonseed oils.—W. Clayton *et al.*, *Nature, Lond.*, ii/1936, 801.

Tea-seed Oil as an Adulterant of Olive Oil has received considerable attention. Tea-seed oil was investigated and figures published in "Tea-seed oil and its uses as an adulterant of olive oil," H. A. Caulkin, *Yearb. Pharm.*, 1927, 616. As a result the following was stated: "The result of our investigation is to show that it is impossible either from a consideration of the available chemical and physical constants of an olive oil, or by means of any of the colour tests hitherto suggested, to ascertain whether such an oil is adulterated with tea-seed oil." This is further confirmed by N. Evers (*Pharm. J.*, i/1933, 195): "It is impossible, therefore, in the present state of knowledge to write a monograph on cod-liver oil or olive oil and give standards which will prevent the addition of adulterants. In these cases we have to rely on the statement at the head of the monograph that the oil is 'the oil obtained from . . .'"

Spectroscopic Detection. The extinction coefficient of olive oil at a wave-length of 268 μ is 11 whilst that of tea-seed oil is 690 and a mixture of olive oil with 5% of tea-seed oil is 46.—S. Judd Lewis, *Analyst*, 1935, 10.

Spectroscopic examination of a number of samples of tea-seed and olive oils showed a considerable variation, which completely vitiates the value of test given by Judd-Lewis.—W. F. Elvidge, *Quart. J. Pharm.*, 1941, 147.

A Colorimetric Method which is specific for tea-seed oil is as follows: Measure into a test-tube 0.8 ml. acetic anhydride, 1.5 ml. chloroform, and 0.2 ml. concentrated sulphuric acid, mix, cool to room temperature and add, by means of a test tube of 4 mm. external and 2 mm. internal diameter, 7 drops of the oil to be tested. If the mixture is cloudy it is cleared by the addition of acetic anhydride drop by drop, with shaking between each addition. After 5 minutes at room temperature the colour is noted. Tea-seed oil gives a deep green by reflected and brown by transmitted light, and olive oil green by reflected and transmitted light, with occasional faint fluorescence. 10 ml. of anhydrous ether is then added from a graduated cylinder and the contents of the tube mixed by one inversion. Tea-seed oil shows a brown colour changing after a minute or so to intense red, reaching a maximum after a few minutes then fading. Olive oil gives an intense green, slowly fading to brown-grey occasionally passing through faint pink. Mixtures show the tea-seed oil colour in proportion to the amount present. By controlling the conditions in ice-water the test may be made approximately quantitative.—J. Fitelson, *J. Ass. off. agric. Chem., Wash.*, 1936, 493. (This method is now official in *Methods of Analysis, A.O.A.C.*, 1940, 440.)

A collaborative study of the Fitelson test (see previous abstract) confirms its accuracy as a method for determining tea-seed oil in olive oil even when the test is carried out by those having no previous experience of it. No red colour is given by any of 9 other seed oils examined.—G. S. Jamieson, *ibid.*, 1937, 418.

Chloroform and acetic anhydride are mixed, cooled in an ice-bath, and 0.8 ml. of a freshly-prepared mixture of 10 ml. glycerin in 100 ml. concentrated sulphuric acid added with constant shaking and cooling. The mixture is left for one hour at room temperature shaking every five minutes until no further separation occurs, cooled to 0° for five minutes and then 3 ml. of water added very slowly. After 5 to 10 minutes a dark green colour appearing in the first stages of the test persists in the case of olive oil but changes to a fugitive dark red at the end in the case of tea-seed oil. Mixtures give intermediate colours, the red-brown being recognisable with 5% of tea-seed oil in olive oil. Strongly rancid olive oil and cottonseed oils give red reactions, and sesame oil, but not arachis oil, may interfere with the test.—W. Siebenberg and W. S. Hubbard, *Quart. J. Pharm.*, 1937, 242.

Fitelson's method may be made quantitative by following the original directions, by cooling the 10 ml. of ether in ice-water and then after mixing the reagents and oil, allowing 4 minutes contact, and finally immersing the tube in ice-water; under these conditions the colour develops more slowly, attaining its maximum intensity after 45 minutes and remaining stable for 5 minutes. Comparison may be made, against a background of white paper, with tubes containing potassium permanganate solution, since it is found that 10% of tea-seed oil gives a colour very close to that of 0.1 ml. of an N/10 potassium

permanganate solution in 100 ml. of water, and 20% corresponds with 0.225 ml. of the permanganate in 100 ml. of solution. On applying the reaction to samples of olive oil of different origin it was found that authentic samples from Tébourba and Ghardinaou in northern Tunisia showed more or less pronounced colour reactions, although all other samples gave negative results. Hence before concluding an olive oil to be adulterated with 10 to 15% of tea-seed oil, the source of the oil should be ascertained.—R. Marcille, *Ann. Falsif.*, 1939, 32, 172.

Some pure olive oils give the colour reaction for cholesterol with acetic anhydride, sulphuric acid and chloroform equivalent to about 10% of tea-seed oil. The test is modified so that tea-seed oil gives a red colour and olive oil a bluish-green colour for all types of the latter. 10% of tea-seed oil can be detected.—W. H. Dickhart, *Amer. J. Pharm.*, 1940, 112, 371.

A modification of the Fitelson test which obviates the positive reaction given by some genuine oils is as follows:—Acetic anhydride 1 ml. is shaken with 2 ml. chloroform and 0.2 ml. conc. sulphuric acid and when the temperature of the mixture is about 40°, 10 drops of oil are added, shaken and then 0.1 ml. syrupy phosphoric acid added. The mixture is kept at 23° to 25° in a water-bath for 10 to 15 minutes and, after addition of 5 ml. of ether (distilled over sodium), is cooled in ice-water until the colour forms. Tea-seed oil gives a red and olive oil a bluish-green colour. The test will detect as little as 10% of tea-seed oil. After the red colour due to the tea-seed oil has disappeared, 1 ml. of acetic anhydride is added and then 20 more of the oil. Conc. sulphuric acid 2.6 ml. is carefully added and the liquid slowly and cautiously mixed. After one minute, the mixture is cooled, 5 ml. of ether are added and the mixture is warmed in water at 25°. The red colour given by tea-seed oil will replace the green first given by the olive oil and will sometimes persist for two hours. The best results are given by reagents free from alcohol and water. The author is of the opinion that oil of pharmacopœial standard should not give a red colour.—W. H. Dickhart, *Analyst*, 1941, 69.

The Iodine Value of the Unsaponifiable Matter (Bolton-Williams number) of oils enables them to be divided into four main groups. Olive oil lies in a group alone and is distinguished from other oils and in particular from tea-seed oil. The unsaponifiable matter is prepared as follows: From 2 to 2.5 g. of oil are saponified by boiling under a reflux condenser with 25 ml. of N/2 alcoholic potash. The solution is titrated with N/2 hydrochloric acid using phenolphthalein as indicator. 5 ml. of N/2 sodium hydroxide is added and the solution is extracted three times or more with 30 to 40 ml. of petroleum ether. The combined extracts are washed with 20 ml. of N/20 sodium hydroxide and then with 20 ml. of water and filtered into a weighed flask, the petroleum ether is distilled off, and the contents dried and weighed. The quantity obtained will vary between 0.01 and 0.04 g. In obtaining the iodine value the Wijs method gives erratic results whilst the Hübl method gives fairly constant figures; the method of Rosenmund and Kuhnhehn is, however, simpler. The N/10 pyridine-sulphate-bromide reagent is made as follows: Mix 8 g. of bromine with 20 ml. of glacial acetic acid and, separately, add gradually 10 g. of concentrated sulphuric acid to 8 g. of pyridine in 20 ml. of glacial acetic acid, cooling during mixing. The two solutions are mixed and made up to 1 litre with glacial acetic acid. The unsaponifiable matter obtained as above is dissolved in 5 ml. of chloroform and sufficient of the pyridine reagent added to leave an excess of about 50%, usually about 10 ml. Allow to stand in the dark for 5 minutes, then add 5 ml. of 10% potassium iodide solution and 40 ml. of water. The liberated iodine is titrated with N/20 thiosulphate solution. A blank experiment is carried out at the same time. The amount of halogen absorbed is calculated as iodine as a percentage of the weight of the unsaponifiable matter, in the same manner as in the Wijs method. The method given above for the preparation of the unsaponifiable matter must be adhered to in order that it may be of the correct degree of purity. The result falls into one of the following groups:—

- | | | |
|----------|--------------------------|--|
| Group 1. | Iodine value 64 to 70. | Animal fats and a few vegetable fats. |
| Group 2. | Iodine value 90 to 96. | Fish and marine animal oils, cocoa butter. |
| Group 3. | Iodine value 117 to 124. | Vegetable oils and fats. |
| Group 4. | Iodine value 197 to 206. | Olive oil only. |

The high value of olive oil as compared with those of oils used as adulterants, mostly in group 3, makes it possible to detect as little as 10%, which will reduce

the value below the minimum.—E. R. Bolton and K. A. Williams, *Analyst*, 1930, 5.

Elaidin Test. A modification of this test in which the elaidin is prepared and then crystallised from acetone, is performed as follows. 10 to 20 g. of the oil is shaken with mercury 0.2 to 0.4 g. and nitric acid 0.5 to 1.0 ml. in a stoppered bottle immersed in water at room temperature for one hour, and then left to stand overnight. The product is dissolved in ether, washed with dilute nitric acid to remove mercury compounds, and then with water until free from acid. The ether and moisture are removed by evaporation and the residue finally heated at 95° for a short time. 5 g. of the dried product is crystallised from 20 ml. of acetone, the solution being kept at room temperature overnight and finally cooled at 0° for two hours. The separated crystals are collected, washed four times with acetone, 5 ml. each washing, dried in a desiccator and weighed. The m.p., iodine value and saponification value are then determined. Olive oil yields 33.1 to 35.5%, m.p. 33.5° to 37.0°, iodine value 67.1 to 68.8, saponification value 196.1 to 197.0. These figures do not differ sufficiently from those of tea-seed oil to enable its presence in olive oil to be detected, but they are widely different from cottonseed oil and show a distinct difference in the saponification value from ground nut oil which is 189.4 to 189.9.—H. N. Griffiths and T. P. Hilditch, *Analyst*, 1934, 312.

Detection of Sesame Oil. A sample of genuine Portuguese olive oil gave a distinct colour with the hydrochloric acid and furfural test of the Belgian Pharmacopœia. Soltsien's test gives a reddish-violet colour with more than 1% of sesame oil. The oil is shaken with an equal volume of a 10% solution of stannous chloride in 38% hydrochloric acid, warming to produce separation.—P. Deltour, *Quart. J. Pharm.*, 1935, 252.

The Detection of Arachis Oil in Olive and Almond Oils.

A modification of the B.P. sorting and confirmatory test which will detect the addition of 5% of arachis oil is performed as follows: 1 ml. of oil is saponified with 5 ml. of 1.5 N alcoholic potassium hydroxide solution by heating on a water-bath for five minutes, avoiding loss of alcohol; 50 ml. of 70% alcohol is added, followed by 0.8 ml. of hydrochloric acid (sp. gr. 1.16). After heating to dissolve any precipitate that may be formed, the solution is cooled in water, stirring continuously with a thermometer, so that the temperature falls at the rate of about 1° per minute. If turbidity appears before the temperature reaches 9°, the usual confirmatory test for arachis oil must be applied; if the oil remains clear at this temperature, arachis oil may be regarded as absent.—N. Evers, *Analyst*, 1937, 62.

The Fluorescence Test. The published statements concerning the nature of the fluorescence of olive oil in filtered ultra-violet light are somewhat contradictory. Crude oils from Spain and Italy give an orange fluorescence whilst the addition of more than 15% of French oil causes a green fluorescence to develop, virgin Apulian oils give lemon-yellow to orange, and refined oils give sky-blue. Oils have been offered in the market giving opaque purple or chocolate coloured fluorescences which may be due to adulterants or refined oil. When genuine virgin olive oil exhibiting a deep golden yellow fluorescence was treated repeatedly with decolorising charcoal (five 1 g. portions per 100 ml. of oil) and filtered, the resulting oil was almost white and showed only the faintest dull blue fluorescence. Arachis, sesame, tea-seed and refined olive oil were bleached, but retained their original blue fluorescence. An oil giving a low Bolton-Williams number invariably exhibits a bright-blue fluorescence after decolorising and should be regarded with suspicion.—T. T. Cocking and S. K. Crews, *Quart. J. Pharm.*, 1934, 531.

An objection to the fluorescence test is that the blue fluorescence, characteristic of refined oils, disappears when pigments such as carotene or chlorophyll are added. The spectra of virgin oils under light produced by a mercury arc shows an intense blue-violet band at 380.0 to 460.0 $m\mu$, a less intense yellow-green band at 510.0 to 590.0 $m\mu$, and an intense red band at 640.0 to 660.0 $m\mu$. If the same oil decolorised or a refined oil is examined, the blue-violet band is very sharp and the yellow is intensified, but the red band is absent.—J. Guillot, *Analyst*, 1935, 432.

Retarding Oxidation. Ultra-violet rays have been found to be mainly the cause of the acceleratory action of light on the oxidation of oil. Deep red cellophane found to give the best protection, followed in diminishing value by orange, violet, grass green and lemon yellow.—H. L. G. Barton and A. Davis, *J. Soc. chem. Ind.*, 1939, 58, 189.

Oleum Rapæ (B.P.C.). Sp. gr., 0.913 to 0.917; $n_{D40^{\circ}}$, 1.463 to 1.467; acid value, not more than 5; saponification value, 171 to 177; iodine value, 97 to 105; unsaponifiable matter, 0.6 to 1.2%.

The B.S.S. No. 631—1935 for rape seed oil gives requirements for specific gravity (0.913 to 0.916), refractive index at 20° (1.4720 to 1.4735), iodine value, saponification value, acidity, unsaponifiable matter, viscosity (Redwood scale, not lower than 390 at 70°F. , or 0.917 poise) and flash point (Pensky-Martens, not below 400°F.).

Determination of Rape and Mustard-Seed Oils. Erucic acid is the characteristic unsaturated acid peculiar to these oils. The percentage of iodine absorption of the solid fraction separated under standard conditions, expressed in terms of erucic acid, may be used as a guide to estimation of purity. 500 to 510 mg. is weighed in a 50 ml. Erlenmeyer flask and saponified with 5 ml. of alcoholic potash (40 ml. of potash solution (sp. gr. 1.5) and 40 ml. of water made up to 1 litre with 95% alcohol) for one hour on a water-bath under an air condenser. The solution is treated with 20 ml. of lead acetate solution (50 g. lead acetate, 5 ml. of 95% acetic acid made up to 1 litre with 80% v/v alcohol), 3 ml. of water and 1 ml. of 96% acetic acid, and the mixture heated under a reflux condenser until the lead salts are dissolved. It is then gradually cooled to room temperature and kept in an incubator at 20° for 14 hours. It is then transferred to a sintered glass crucible (3G/10, Schott, and Gen) by means of 70% alcohol (cooled to 20°) and the precipitate washed with 12 ml. (in small portions) of the cooled alcohol that had previously been used for rinsing the flask. The precipitate is dissolved in 20 ml. of a hot mixture of equal parts of 95% alcohol and 96% acetic acid in a tall 150 ml. beaker, covered with a watch glass. The warm solution of lead salt and the crucible are washed into a 350 ml. glass-stoppered Jena glass bottle with 10 ml. of mixed alcohol and acetic acid. The iodine value of the lead salt is then determined by the method of Margosches, Hinner and Friedmann, *Z. angew. Chem.*, 1924 37, 334, in which 20 ml. of freshly prepared N/5 alcoholic iodine solution is added, shaken with 200 ml. of distilled water and kept in the dark for one hour, and finally titrated with N/10 thiosulphate solution. A blank of 30 ml. of mixed acetic acid and alcohol is titrated and used as a correction. The "value" is the percentage of iodine absorption in terms of erucic acid, 1 ml. of N/10 thiosulphate = 16.9 mg. of erucic acid. The following values were found:—

| | |
|---|--------------|
| Oil of <i>Brassica juncea</i> | 43.8 to 46.9 |
| Oil of <i>Brassica napus</i> | 45.7 to 47.2 |
| Oil of <i>Brassica campestris</i> | 43.8 to 48.8 |
| Arachis Oil | 1.32 to 1.7 |
| Linseed Oil | 1.6 to 1.8 |
| Sesame Oil | 1.8 to 2.1 |
| Niger-seed Oil | 2.2 to 2.4 |
| Cottonseed Oil | 1.4 to 1.7 |
| Castor Oil | 0.2 to 0.3 |

—S. Neorgi, *Analyst*, 1936, 597.

A method almost identical with the above is used for the estimation of rape oil in edible oils. The presence of 5% or less of rape oil may be detected by absorption of the lead salt upon lead palmitate. For this purpose 0.25 g. palmitic acid is mixed with 0.5 g. of the oil to be tested and 7.5 ml. of the alcoholic potash used for saponification. To the soap solution are added 2 ml. of 96% acetic acid, 30 ml. of alcoholic lead acetate solution and 5 ml. of water. The mixture is warmed, allowed to stand overnight and the precipitate filtered off and treated as above.—J. Grossfeld, *Analyst*, 1936, 124, and 1937, 561.

The characteristic sterol of rape seed oil is brassicasterol, m.p. 148° , which has been shown to consist of 7:8-dihydroergosterol.—E. Fernholz and H. E. Staveland, *J. Amer. chem. Soc.*, 1940, 1875.

Turtle Oil. The commercial oils are generally obtained from the muscles and genital glands of the giant sea-turtle. There are several varieties of oil on sale. A bulk of four separate samples of West African oil had the following characters:—Solid at ordinary temperature, light yellow colour, mild but distinct odour, melted to a golden-yellow coloured oil. It bleached on exposure to light. M.p., 24.6° incipient fusion, 25.6° final fusion; solidifying point, 22.5° ; titre, 25.5° ; sp. gr., 0.9112/40°; $n_{D40^{\circ}}$, 1.4599; saponification value, 209; unsaponifiable matter (S.P.A. method), 0.6%; iodine value of unsaponifiable

matter (Bolton-Williams method), 92.5; iodine value, 64.6; acid value, 2.0; percentage of insoluble bromides on the free acids (Gemmel's method), 5.0; m.p. of the bromides, darken above 200°; Reichert-Meissel value, 0.2; Polenski value, 1.7; Kirschner value, 0.06; acetyl value, 3.5. Other samples of oil gave the following figures:—

| Variety of Oil | $n_{D_{40}^{\circ}}$ | Iodine value | Sap. value | Unsap. matter Pet. ether extract |
|---|----------------------|--------------|------------|--|
| Specially processed and deodorised | 1.4599 | 66.6 | 214.0 | 0.44% |
| Egyptian Oil | 1.4585 | 57.2 | 213.5 | 0.30% |
| African Oil, same source as above | 1.4599 | 67.8 | 209.0 | 0.43% |

The Egyptian oil has the greater sale in this country and is supposed to be obtained from the liver and glands.—W. Lee, *Analyst*, 1935, 650.

A specimen of green turtle oil from *Chelonia japonica* Thunbery, from the Bonin islands, an orange-yellow liquid with a deposit at ordinary temperature. Very little colour was given with the antimony trichloride test; sp. gr. at 20°/4°, 0.9150; $n_{D_{20}^{\circ}}$, 1.4662; saponification value, 205.8; iodine value (Wijs), 64.1; acid value, 1.36; unsaponifiable matter, 0.52%. The fatty acids converted to methyl esters, fractionally distilled and the fractions examined for their component acids gave, as chief constituent, oleic acid, with myristic, palmitic and stearic acids also present together with a small proportion of the highly unsaturated acids C_{20} and C_{22} . The oil differs from most marine animal oils in the comparative lack of C_{20} and C_{22} acids.—M. Tsujimoto, *Perfum. essent. Oil Rec.*, 1937, 273.

Other figures are given for oil of green turtle by C. Hata (*Analyst*, 1939, 64, 610). Physical and chemical properties and vitamin content have been examined and published in *J. Amer. pharm. Ass.*, 1940, 437.

Oleum Maydis (U.S.P. XI). Sp. gr. at 25°, 0.914 to 0.921; saponification value, 188 to 193; iodine value, 112 to 128; unsaponifiable matter, not more than 2%.

The British Standard Specification for crude maize oil obtained from maize germ, *B.S.S. No. 651*—1936, does not provide for decomposed oil recovered after a fermentation process and known as "Fermentation Maize Oil." The specification includes requirements for colour, specific gravity, refractive index, iodine value, saponification value, acidity and unsaponifiable matter.

OLEUM RICINI

Oleum Ricini (B.P.). Sp. gr., 0.958 to 0.969; $n_{D_{40}^{\circ}}$, 1.4695 to 1.4730; acid value, not more than 4.0; saponification value, 177 to 187; iodine value, 82 to 90; α_D , not less than +3.5. Remains bright when kept at 0° for 3 hours. **Oleum Ricini, U.S.P. XII**, has a sp. gr. of 0.945 to 0.965 at 25°; saponification value, 179 to 185; iodine value, 83 to 88.

Microscopic Identification of Castor Oil. A small drop of the oil is mixed with an equal quantity of a saturated solution of potassium hydroxide in *n*-butyl alcohol. The preparation is examined at a magnification of 430; characteristic crystals are formed, for microphotograph of which see *Amer. J. Pharm.*, 1937, 67.

The **Optical Rotation** of commercial varieties of castor oil varies within wide limits; that of inferior qualities, obtained by extraction of the cake with carbon disulphide, is extremely high. It has been suggested that this is due to auto-esterification of ricinoleic acid, the rotatory power of these condensation products increasing with the number of molecules of the acid which combine.—M. Roy, *Quart. J. Pharm.*, 1933, 596.

The **Solubility** of castor oil in alcohol decreases with age. Failure of the oil to pass solubility tests in alcohol of less strength than 95% should not be considered proof of adulteration.—H. P. Trevithick and M. F. Lauro, *J. Soc. chem. Ind., Lond.*, 1929, 528.

Effect of Heating on Rotatory Power of Castor Oil. The B.P. '14 solubility test in petroleum ether is unsatisfactory and depends on the latter containing some aromatic hydrocarbon. When petroleum ether (b.p. 50°/60°) containing less than 0.2% of aromatic hydrocarbon was used for the B.P. '14 test a clear mixture was obtained at 15.5° which was turbid on the addition of 3 vols. of the petroleum ether and on warming only cleared at 26.9°. The addition of as much as 4% of benzene was necessary before the petroleum ether would give a clear solution under the B.P. conditions at 21°.—T. T. Cocking and S. K. Crews, *Quart. J. Pharm.*, 1929, 217.

The B.S.S. No. 650—1936 for castor oil (Firsts' Quality) does not apply to oil which is intended for medicinal use and is included in the B.P. It includes a "cold test" for stearine, and requirements regarding colour (not deeper than a colour equivalent to 2.2 yellow and 0.25 red Lovibond units), sp. gr. (0.958 to 0.969), refractive index at 20° (1.477 to 1.481), iodine value (82 to 90), saponification value (177 to 187), solubility in alcohol at 0°, acidity (not exceeding the equivalent of 2.0% of oleic acid), unsaponifiable matter (not more than 1%) and acetyl value (not below 144). The specification does not include standards for optical rotation or viscosity.

OLEUM SESAMI

Oleum Sesami (B.P.). Sp. gr., 0.921 to 0.924; $n_{D40^{\circ}}$, 1.4650 to 1.4665; acid value, not more than 4.0; saponification value, 188 to 193; iodine value, 103 to 112. Complies with tests for absence of cottonseed oil and of arachis oil. The test for absence of sesame oil in other oils consists in agitating 2 ml. of the oil with 1 ml. of a 1% w/v solution of sucrose in hydrochloric acid and standing for 5 minutes, when the acid layer should not appear pink. **Oleum Sesami, N.F. VII**, has a saponification value of 188 to 193; iodine value, 103 to 115; sp. gr. at 25°, 0.916 to 0.921.

The B.S.S. No. 656—1936 for sesame oil does not apply to medicinal oil included in the B.P. The specification includes the usual tests for other oils and requirements for specific gravity, refractive index, iodine value, saponification value, acidity and unsaponifiable matter.

Oleum Sojæ (B.P.C.). Sp. gr., 0.924 to 0.927; $n_{D40^{\circ}}$, 1.4675 to 1.4685; acid value, not more than 5; saponification value, 190 to 194; iodine value, 130 to 137; unsaponifiable matter, 0.7 to 1.5%.

The B.S.S. No. 653—1936 for crude soya bean oil includes requirements regarding colour, sp. gr. (0.924 to 0.928), $n_{D10^{\circ}}$ (1.473 to 1.477), iodine value (129 to 141), saponification value (not below 190), acidity (not more than the equivalent of 1.5% of oleic acid), and unsaponifiable matter (not more than 1.2%).

Detection of Linseed Oil. For pure soya bean oil, $\frac{I-126.19}{H}$ is greater than 11, where I = the iodine value and H is the insoluble bromine value determined as follows:—2 g. of oil which has been made neutral by shaking with 10% sodium hydroxide, and filtering, is dissolved in 40 ml. ether and 5 ml. glacial acetic acid, bromine 0.8 ml. is added drop by drop from a burette, and the mixture kept at 0° for 3 hours. The precipitate is filtered off in a tared glass crucible, washed with four 10 ml. portions of ether at 0° and dried at 100° till of constant weight. The percentage result = H . The value for H in an oil may fall from about 0.55 to 0.20 during the course of a year.—J. F. Carriere, *Analyst*, 1930, 64.

Detection of Soya Bean Oil in Nut and Olive Oils. Shake 10 ml. of oil and 1 ml. of nitric acid together, place in a water-bath for 15 minutes and then allow to cool. In the case of olive and nut oils the mixture solidifies completely, soya bean oil gives a viscous, reddish-brown mixture, the coloration of which is sufficient to enable the presence of 10% to be seen in either of the preceding oils. The iodine values of the three oils are, nut oil, 84 to 105; olive oil, 78 to 95; soya bean oil, 130 to 142.—A. Richard, *Ann. Falsif.*, 1928, 21, 579, per *Quart. J. Pharm.*, 1929, 103.

OLEUM THEOBROMATIS

Oleum Theobromatis (B.P.). M.p., 30° to 35°. n_{D40}^{20} , 1.4565 to 1.4575. Acid value, not more than 4.0. Saponification value, 188 to 195. Iodine value, 35 to 40. The *U.S.P. XII* specifies a refractive index at 40° of 1.4537 to 1.4578 and an iodine value of 35 to 40. (The m.p. and saponification value limits are the same as the B.P. '32.) Solidification point of the fatty acids, 45° to 50°.

Genuine cocoa-butter extracted from five varieties of cocoa had a "crystallisation temperature" of 20.0°; Borneo tallow has a "crystallisation temperature" of about 31.3° and the presence of the latter in cocoa-butter can be shown by means of a special apparatus devised for determining this factor.—S. A. Ashmore, *Analyst*, 1934, 515.

Extracted Cocoa Butter. A large amount of cocoa butter is made by extracting with benzene the waste products (shell and broken seeds) of cocoa and chocolate manufacturing. The characteristics of such cocoa butter are pale yellow tending to grey in colour; m.p., 28° to 30°; acid value, 1.5 to 2.5; iodine value, about 38; saponification value, 193; tests for foreign fats negative.—Bodinus, *Quart. J. Pharm.*, 1929, 438.

Distinction between Pressed and Extracted Cacao Butter. 2 g. of the butter is dissolved in 5 ml. of chloroform in a dry test-tube and is gently mixed with 5 ml. fuming hydrochloric acid (1.192, about 37%). Extracted cocoa shell butter gives a lower layer pale green at first and dark green after a minute. When heated to 50° for 2 minutes with 2 drops of concentrated nitric acid (1.42) it becomes reddish brown with a violet tinge. In the case of expressed butter the mixture remains colourless. This reaction detects about 20 to 25% of added extracted cocoa butter. No colour is given by ordinary or hardened coconut fat, palm kernel fat, hardened whale oil, vegetable hard butter ("Cocola" and "Kernel"), French vegetable fat ("Banka") or English hard butter ("Mikon"). If 5 drops of sulphuric acid (1.84) are used in place of the 5 ml. of hydrochloric acid, as above, the mixture becomes at once deep violet changing to brownish-violet after 2 minutes in a water-bath at 50° for extracted oil, whilst with mixtures of expressed and extracted oils the colour is pale brown in the cold, deep brownish violet at 50°. Expressed butter gives a colourless mixture cold and a reddish-yellow with violet tinge at 50°. About 10% of extracted butter is detectable.—Aufrecht, *Analyst*, 1929, 346.

Aufrecht's tests may sometimes give misleading results according to the nature of the manufacturing process and the amount of shell present. If 0.5 to 1 g. of the fat is melted in a test-tube and shaken with 3 ml. of glacial acetic acid, and when the acid layer has separated it is observed in filtered ultra-violet light, a bright green-yellow fluorescence is obtained from solvent extracted products while expressed oils give no colour. Molin's antipyrine test may be used by mixing 0.5 g. of butter, 2 ml. of 95% alcohol, 3 ml. of hydrochloric acid (1.19) and a few crystals of antipyrine, boiling and allowing to cool. The liquid layer develops a red colour. These reactions are not given by coconut oil or palm oil.—A. Castiglioni, *Analyst*, 1935, 257.

Examination in Ultra-violet Light. 0.1 g. is weighed into a thin-walled test tube and dissolved in 10 ml. of petroleum ether and examined in ultra-violet light. A non-fluorescent butter is almost certainly not solvent extracted. A strongly fluorescent sample is either solvent extracted or contains mineral oil. Mere traces of mineral oil such as may occur from factory waste give rise to strong fluorescence. With slight fluorescence no definite conclusion can be drawn.—W. T. Field, *Analyst*, 1930, 744.

Detection of Coconut and Palm Kernel Oils. These tests depend upon the complete insolubility of cacao butter soap in sodium chloride solution. Saponify about 2.5 g. of the cacao butter with alcoholic potash, drive off the alcohol and dissolve the soap in 50 ml. of hot water, allow to cool, add 50 ml. of saturated sodium chloride solution, agitate frequently during fifteen minutes and then filter. A further 50 ml. of saturated sodium chloride solution is added to the filtrate. The presence of coconut or palm-kernel oils is indicated by more than a slight opalescence.—F. Strube, *Yearb. Pharm.*, 1909, 20.

Saponify 5 g. of the sample with 10 ml. of alcoholic potash (25 g. of KOH in 200 ml. of alcohol), evaporate the alcohol, add 5 ml. of water and again evaporate, dissolve in 10 ml. of water and add with stirring 100 ml. of saturated sodium chloride solution, allow to stand 15 minutes, with occasional agitation and filter on a Buchner funnel. To 100 ml. of the filtrate add with stirring 100 ml. of saturated sodium chloride solution, allow to stand 15 minutes and again filter. Render the filtrate slightly acid with hydrochloric acid; a turbidity or milkiness indicates the presence of coconut or palm kernel fat in the sample.—W. F. Baughman, *Yearb. Pharm.*, 1926, 126.

Detection of Hardened Fats. A method depending on the fractional crystallisation of the lead salts of the fatty acids is as follows:—2.5 g. of fat is saponified with 1 ml. of 50% potash and 25 ml. of 95% alcohol. The soap solution is treated with 100 ml. of lead acetate solution (50 g. of lead acetate, 5 ml. of acetic acid 96%, and alcohol 80% to make 1 litre). The salts are completely dissolved by warming, 20 ml. of boiling water is added, and the mixture set aside overnight. Collect the precipitate in a glass Gooch crucible and wash with 50 ml. of alcohol 70%. The crucible is then fitted in an inverted position in an extractor, 3 ml. of acetic acid 96% is poured over the plate and the salts dissolved by extraction in 100 ml. of the lead acetate solution; after the addition of 15 ml. of hot water and agitation the mixture is set aside overnight. The lead salts are again collected, dissolved in 5 ml. of acetic acid and 10 ml. of alcohol 96% and then decomposed whilst warm with 5 ml. of nitric acid (1 : 2) in a flask filled to the neck and warmed so that the fatty acids separate as a clear layer. When cold the acid cake is removed, washed with cold water, dried in a steam oven, returned to the dried-out flask, dissolved in chloroform and used for the determination of the iodine value. The result is expressed in terms of *isooleic* acid in the original fat. One ml. of N/10 thiosulphate = 0.565% of *isooleic* acid. The presence of as little as 9.3% of hardened arachis oil in cacao butter can be detected.—J. Grossfeld, *Quart. J. Pharm.*, 1930, 643.

OPIUM

Opium (B.P.). Contains in its moist state as imported not less than 9.5% of morphine calculated as anhydrous. Assayed by the B.P. method: 8 g. mixed thoroughly in a mortar with 30 ml. of water and 2.0 g. of calcium hydroxide is transferred to a tared flask, with water to 90 g.; after shaking for 30 minutes, the mixture is filtered and 52 ml. of the filtrate (equivalent to 5 g. of the opium) shaken with 5 ml. of alcohol and 25 ml. of ether, 2.0 g. of ammonium chloride added, shaken for 5 minutes and occasionally during 30 minutes, the total time of shaking being 15 minutes, and stood overnight; the ethereal layer is decanted on to a plug of cotton wool in a funnel, the flask and contents and filter rinsed with 10 ml. of ether, and the filter with a further 5 ml. in small portions; the aqueous liquid is then poured through the filter, and the flask and filter washed with morphinated water till free from chloride; the crystals on the filter are washed back into the flask, boiled with excess N/10 sulphuric acid, and the cooled liquid titrated with N/10 sodium hydroxide to cochineal or methyl red, a correction of 0.052 being added.

Opium, *U.S.P. XII*, should yield not less than 9.5% of anhydrous morphine. It is assayed by a similar process on the water-soluble matter, finally treating the crude morphine on the filter with hot neutral methanol. The hot solution of morphine in methanol is cooled and excess of N/10 sulphuric acid is added. The solution is then diluted with distilled water and boiled carefully to remove the methanol, since the titration end-point to methyl red is not very delicate in its presence. The amounts of methanol specified in the *U.S.P. XI* were not always sufficient to dissolve all the morphine in the crude precipitate, and the *U.S.P. XII* uses a larger quantity. No correction for the solubility of morphine is allowed. The *U.S.P. XI* process gave results showing as much as 0.75 to 1.5% less morphine than the *U.S.P. X* method, and on the average for high testing opiums, about 1.5% less morphine than the *B.P.* method. The assay process of the *Fr. Cx.* 1937 is similar to the International process given below. The concentrated extract is also assayed for other alkaloids by extraction with ether-chloroform from sodium hydroxide liquid; morphine content 50% and other alkaloids 30%.

League of Nations' Process. A report of a Commission of Experts for the Standardisation of Methods for Determining the Morphine Content of Raw Opium described an assay process which was published, with some comments by Prof. L. van Itallie, in the *Bulletin de la Fédération Internationale Pharmaceutique*, 1933, 14, 98.

The experts' method has now been published by the Health Committee of the League of Nations and is preceded by a method of sampling, the practical utility of which has yet to be established.—*Bull. Hlth Org. L. o. N.*, 1938, 429.

The determination is carried out in three parts. (1) Determination of the loss of weight on drying at 103° to 105° (moisture). (2) Determination of the amount of extractive soluble in water in the presence of calcium hydroxide under the conditions of the process. (3) Determination of the morphine content.

Determination of the Loss in Weight on Drying (Moisture). Weigh 1 g. of opium (to within ± 5 mg.) in a weighing-bottle with a ground-in stopper; heat for two hours at 103° to 105° and weigh. Continue heating until the loss in weight after 1 hour in the oven is not more than 0.005 g. The loss in weight is calculated on 100 g. of the opium being analysed and in the following formula this percentage loss is denoted by the letter *F*. When dealing with soft opium the mass is diluted with a little water and dried in a thin layer.

Determination of the Extractive and of the Morphine. Triturate in a mortar 4 g. opium (weighed to within ± 5 mg.) with 1 g. of calcium hydroxide and 10 ml. of water so as to produce a homogeneous mixture. Dilute with another 10 ml. of water and set aside the mixture for 15 minutes, stirring frequently. Then by means of small quantities of water transfer the mixture to a small tared flask and add water until the contents of the flask weigh 45 g. (weighed to within 0.1 g.). Cork the bottle and shake vigorously and continuously for 30 minutes. Pour out the contents of the bottle on to a glass filtering funnel No. 3G3 of Schott and Gen, Jena, or on to a filtering funnel of similar pattern but having the same porosity and suitable dimensions. The liquid is at first allowed to flow freely, then the slightest possible suction is applied. Part of the filtrate is used to determine the extractive and part for the determination of the morphine.

A. DETERMINATION OF THE CONTENT OF EXTRACTIVE. Concentrate on a water-bath 3 g. of the filtrate (weighed to within ± 0.1 g.), then dry the remainder at 103° to 105° until the loss in weight after drying for one hour is not greater than 0.003 g. The weight of the remainder, in mg., is used for determining the extractive (*E*) given by 100 g. opium, according to the following formula:—
$$E = \frac{(1000 + F) M}{3 - M}$$
 where *M* denotes the weight in grammes of the residue from 3 g. of the filtrate and *F* the percentage of moisture in the opium.

B. DETERMINATION OF THE MORPHINE CONTENT. In a 50 ml. Erlenmeyer flask, or in some other suitable vessel, weigh 25 g. of filtrate (to within ± 0.1 g.) and add 2.5 ml. of alcohol (90%) and 12.5 ml. of ether. Close the flask, shake it to mix the liquids and add 1 g. of ammonium chloride. Shake well for 5 minutes, then frequently during 30 minutes. Set aside the mixture in the closed flask until the next day. Shake well to detach the precipitated morphine and pour the contents of the flask as completely as possible on to a filtering funnel No. 3G4 of Schott and Gen, Jena, or on to a filtering funnel of similar pattern, but having the same porosity and suitable dimensions. Avoid wetting the upper part of the funnel. Filter the liquid completely with the help of slight suction, then wash the flask with 3 ml. of ether; pour the latter on to the filtering funnel and without using suction wash it by inclining and shaking, then filter the ether completely by suction. The washing of the flask and the filtering funnel is repeated in the same way, each time with 3 ml. of water saturated with morphine, until the filtrate gives no reaction for chlorides. Heat the flask, in which there may still remain a little morphine, and the glass filtering funnel containing the greater part of the morphine, for 30 minutes at a temperature of 103° to 105° . After cooling, grease the upper and inner part of the filtering funnel to a depth of 0.5 cm. with soft paraffin; then fix it by means of a cork into a 300 cm. filtering flask. Warm 10 ml. of methyl alcohol in the flask (preferably with a reflux condenser because of the toxicity of the vapour of methyl alcohol) to dissolve the remaining crystals of morphine attached to it; pour the warm solution on to the filtering funnel without applying suction, dissolve the greater part of the morphine by shaking, and filter the solution using suction. This operation is repeated twice, using each time 10 ml. of methyl alcohol. Then, by means of a small wash-bottle rinse all the deposits of morphine which may have formed on the filtering funnel and on the lower tube with 10 ml. of methyl alcohol; pour this alcohol into the filtering flask. The impurities in the morphine will thus remain on the plate of the filter. Verify whether the filtrate is quite clear; should a little morphine be precipitated dissolve it by warming slightly. Add to the clear liquid 5 to 10 drops of methyl red and titrate with N/10 hydrochloric acid or N/10 sulphuric acid to a faint orange. Dilute the liquid with 120 ml. of freshly boiled and cooled water, which changes the colour of the solution to yellow; and complete the titration by adding N/10 acid until the liquid begins to turn red.

The morphine content is calculated according to the following formula:

$$(a) \text{ in \% of anhydrous opium } \frac{(1000 + E + F)(A + 1) \cdot 0.114}{100 - F}$$

$$(b) \text{ in \% of original opium } \frac{(1000 + E + F)(A + 1) \cdot 0.114}{100}$$

E = percentage yield of extractive in the presence of calcium hydroxide, calculated on the opium.

F = percentage of moisture in the opium.

A = ml. of N/10 acid used in the titration.

This formula allows for a correction of 1 ml. of N/10 acid (equivalent to 0.0285 mg.) for the morphine remaining in solution.

Some Defects in the International Process. The process is undoubtedly superior to many published methods, and particularly to the official methods of most foreign pharmacopœias. It is, however, much more complicated and troublesome to perform than the method of the British Pharmacopœia and it is extremely doubtful whether it is superior or even equal to the latter method as regards accuracy.

Its principal disadvantages are:

(1) The very small weight of opium used for the test.
 (2) The unnecessarily involved practical details, some of which could quite well have been dispensed with without appreciably diminishing the accuracy of the results.

(3) The decidedly clumsy manipulation, such as the weighing of all liquids, the method adopted for the initial filtration and the directions for the collection, washing and solution of the morphine. Some of these defects cannot fail to cause very appreciable errors.

If the process is performed exactly as described, it appears very improbable that close agreement between different analysts using the method will occur. In order to produce a really accurate method capable of giving reasonably consistent

results in different hands very considerable modifications would be needed, some of which might very well be in the direction of simplification.

Observations on the opium assay.—J. Rosin and C. J. Williams, *J. Amer. pharm. Ass.*, 1935, 1953.

Proposed Alternative Assay Process. The following method represents an attempt to eliminate sources of error in previously published methods due to incomplete extraction of the opium, inaccurate calculations of the aliquot proportions, incomplete precipitation of the morphine and its contamination with other substances.

3 g. of finely powdered sample is heated for 10 minutes on a boiling water-bath with 50 ml. of N/20 sulphuric acid, stirring to produce a uniform suspension, and 8 g. of anhydrous sodium sulphate is dissolved in the mixture which is then cooled and allowed to stand for 30 minutes. The mixture is filtered through filter paper supported on a 3G3 glass filter, and the filter washed with 3 ml. of a saturated solution of sodium sulphate in N/20 acid. The filtrate is shaken vigorously with 5 ml. of 4N sodium hydroxide. The residue on the filter is heated with 30 ml. of N/20 sulphuric acid for 5 minutes, treated with 4.5 g. of anhydrous sulphate, cooled, filtered, and the filter washed as before. If the opium is rich in morphine, three extractions should be carried out using 45, 20 and 15 ml. of acid respectively with corresponding amounts of sodium sulphate. The mixed filtrates are diluted to 90 ml., shaken well and allowed to stand for 30 minutes and then filtered through a thick folded filter paper. The filtrate is shaken with 80 ml. of benzene, and after separation, 75 ml. of the aqueous extract (= 2.5 g. of sample) is pipetted off and shaken vigorously for 1 minute with 2 g. of sodium bicarbonate and 75 ml. of a warm mixture of chloroform and alcohol (3:2). The chloroformic extract is run off into a second separator and treated with 25 ml. of pure chloroform and 2 ml. of water, and the flask rotated without shaking and the aqueous layer rejected. The extraction is repeated with 30 and 15 ml. of warm chloroform-alcohol mixture and the chloroformic extracts run off into the second separator. The mixed chloroformic extracts are allowed to stand 30 minutes and then filtered through cotton wool which has been covered with a little anhydrous sodium sulphate, the filter being rinsed with two quantities, each of 5 ml., of chloroform. The chloroform is evaporated and the residue is warmed on a water-bath with 1 ml. of alcohol and 25 ml. of benzene until almost completely dissolved. 20 ml. of N/2 sodium hydroxide is added, followed by 1.5 g. of ammonium sulphate, and the mixture is shaken vigorously for 2 minutes and then, after 12 hours, filtered through a 3G3 filter, the flask and filter being rinsed with 2 ml. of benzene, then three times with 5 ml. of water saturated with morphine, and finally with 3 ml. of ether. The morphine in the flask is dissolved in 15 ml. of N/10 hydrochloric acid, warmed on a water-bath, and the solution transferred quantitatively to the filter, any morphine still remaining being dissolved by the further addition of 5 to 10 ml. of the acid. Flask and filter are thoroughly washed with recently boiled and cooled water. The solution is titrated to the yellow colour of methyl red with N/10 alkali and then with N/10 acid until the red colour begins to return. To the volume of acid used by the alkaloid 0.4 ml. is added to correct for the morphine remaining in the mother liquor. 1 ml. of N/10 acid corresponds to 0.0285 g. of anhydrous morphine.—E. Knaff-Lenz, *Pharm. Monatschef.*, 1937, 17.

Eder and Wackerlin's Method (*Quart. J. Pharm.*, 1937, 680). Carefully triturate 1 g. of opium with 1 ml. of water in a 30 ml. mortar, having a roughened inner surface, until homogeneous. Mix with 1 ml. of water and 0.4 g. of calcium hydroxide and then gradually add 8 ml. of water. Filter directly into a 150 ml. separating funnel through a 3G3 or 3G4 sintered glass filter using slight suction. Rinse the pestle and mortar with 7 ml. of water, pour on to the filter with the suction discontinued, rub the opium to a smooth paste, filter, and repeat the procedure five times. To the filtrate add 0.3 g. of ammonium chloride and shake for 1 minute with 60 ml. of chloroform-isopropyl alcohol mixture (3+1). Allow to separate and then stand for 10 minutes, filter through a double filter wetted with the solvent mixture into a 250 ml. separating funnel. Repeat the extraction twice using 40 and 30 ml. of solvent mixture, and finally wash the filter with a further 15 ml. Shake the mixed filtrates with 20 ml. of N/10 sodium hydroxide for 1 minute, draw off the chloroform-isopropyl alcohol layer and pour off the alkaline layer through the neck of the funnel, rinsing the neck with a few drops of water. Return the chloroform-isopropyl alcohol solution to the funnel and repeat the procedure twice using 15 and 10 ml. of

N/10 sodium hydroxide, finally washing the funnel with 3 ml. of water and adding to the alkaline liquid. To the latter is added 0.5 g. of ammonium sulphate and the liquid extracted three times with solvent mixture using quantities of 60, 40 and 30 ml., filtering each time and using 15 ml. of solvent mixture to wash the filter. Evaporate the solution to 10 ml., transfer to a 50 ml. stoppered flask using three quantities, each of 5 ml., for rinsing, and evaporate the solvent at 80° in a current of air. Cool the residue, add 1 ml. of alcohol 95%, 10 ml. of N/10 sodium hydroxide and 5 ml. of ether, stopper, and shake until dissolved. Add 0.4 g. of ammonium chloride and shake until the morphine begins to precipitate and for 5 minutes longer. Allow to stand overnight, noting the temperature. Shake, cool in cold water, filter through a sintered glass funnel, washing flask and funnel successively with 2 ml. of ether and then four quantities each of 2 ml. of morphine-saturated water. Dissolve the morphine in 15 ml. of methyl alcohol, added in 3 ml. portions and washing the lower part of the funnel with the last 3 ml. Titrate to methyl red with N/10 acid, add 45 ml. of water and complete the titration. Deduct any acid used in a blank test on the methyl alcohol and add to the weight of morphine thus determined a correction depending on the temperature at which the morphine stood overnight. At 10° the correction is 0.0053 g., at 20° 0.0062 g., and at 30° 0.0080 g. Two variants of this method are also described.

The method is modified to remove substances which prevent the precipitation of morphine. A shorter variation of this improved method is given. The extraction of the opium is modified by triturating the paste, prepared from 5 g. of the drug and 5 ml. of water, with a further 5 ml. of water and 3 g. of manganese sulphate prior to the addition of the calcium hydroxide. In the new method 3 g. of calcium hydroxide are used. 50 ml. of the extract so prepared are washed with 60 ml. of a mixture of equal volumes of benzene and carbon tetrachloride, 0.4 g. of ammonium chloride is added and the solution shaken with 60 ml. of a mixture of 3 volumes of chloroform and 1 volume of isopropyl alcohol for one minute. After separation the chloroform-isopropyl alcohol layer is filtered through an adsorption filter, consisting of a tube 20 cm. long and 17 mm. internal diameter, which is packed with a plug of cotton wool moistened with chloroform-isopropyl alcohol, 5 g. of alumina (washed into the tube with chloroform-isopropyl alcohol) and finally a layer of filter paper. The extraction is repeated with 40 and 30 ml. portions of chloroform-isopropyl alcohol and the extracts also passed through the adsorption filter. The latter is then washed with three 10 ml. portions of chloroform-isopropyl alcohol. The filtrate is evaporated to 10 ml., placed in a 50 ml. flask and the solvents completely removed by heating to 75°. The crude morphine is then purified and titrated as described originally. According to the temperature at which the morphine is allowed to stand, the following correction is added: 10°, 0.0064 g.; 20°, 0.0073 g.; 30°, 0.0091 g. of morphine. Finally, methods are given for the homogenisation of opium samples and for the determination of water in them. —R. Eder and E. Wäckerlin, *Pharm. Acta Helvet.*, 1940, 15, 227.

Varieties. Turkey opium is produced in Asiatic Turkey and occurs in rounded, conical, irregular or flattened masses usually enveloped in poppy leaves, and sometimes more or less covered with the winged fruits of a species of *Rumex*. The weight of the cakes varies from about 1 oz. to several pounds, the majority of the pieces varying from about 1 lb. to 4 lb. Turkey opium includes the varieties, "Soft Shipping" opium and "Druggists" opium. It is now exported in cases containing usually 40 pieces of more uniform consistency and shape, each piece weighing about 4 lb. or 4 kilos. European opium is produced chiefly in Bulgaria, Greece and Yugoslavia; it resembles the "Soft Shipping" variety of Turkey opium in its general characters. Yugoslavian monopoly opium occurs in fairly uniform flattish and elongated oval masses, each weighing about $\frac{3}{4}$ or 1 lb. Persian opium occurs in brick-shaped masses weighing about 1 lb., usually wrapped in red paper and tied with red string. It formerly contained varying proportions of certain native gums added during manufacture to obtain a product suitable for moulding, but this addition is now prohibited. Indian medicinal opium occurs in square blocks weighing about 2 lb. and is wrapped in white paper. Bulgarian, Russian and Syrian opiums are occasionally exported.

Constituents. Opium contains morphine in proportions varying from 5 to 21% of the dried opium. The morphine exists in combination with meconic and sulphuric acids in the form of salts readily soluble in water. The "Soft Shipping" variety of Turkey opium contains about 15 to 18% of morphine,

while "Druggists" opium of good quality contains about 12 to 16%. Dried European opium contains from about 15 to 21% or occasionally more. Persian opium contains about 10 to 12% and occasionally as much as 13.5%. Undried Indian medicinal opium has varied considerably at different times, sometimes containing as little as 7%, and sometimes as much as 12%. Narcotine ranges from 1.5 to 12.5%. Codeine exists to the extent of 0.3 to 4%, Indian opium containing the highest proportion and Turkey opium the lowest. The remaining alkaloids constitute about 1% of the drug. They include thebaine, narceine, papaverine, meconidine, codamine, laudanine, laudanoline, neopine, lanthopine, protopine, cryptopine, rhœadine, oxynarcotine, pseudo-morphine, gnoscopine, xanthaline (papaveraldine), tritopine, hydrocotarnine, porphyroxine (in Indian opium) and possibly others. Meconin, meconoidin and opionin are indifferent substances, present in small proportions only. Other constituents are mucilage, sugar, wax and rubber, together with salts of calcium, magnesium and potassium.

Determination of morphine, narceine, narcotine, papaverine, codeine and thebaine in opium.—B. Kljotschkina, *Arch. Pharm., Berl.*, 1933, 271, 558, per *Quart. J. Pharm.*, 1934, 119.

Opium Pulveratum (B.P.). Adjusted with lactose to contain from 9.5 to 10.5% of morphine, calculated as anhydrous. Opium Pulveratum, *U.S.P. XII*, yields from 10 to 10.5% of anhydrous morphine.

Extractum Opii Siccum (B.P.). Contains 19 to 21% of morphine, calculated as anhydrous. Assayed by trituration of 4 g. with 5 ml. of water and then 20 ml. of water and 2 g. of calcium hydroxide, finally adjusting with water to 86 g. The assay is then completed as for Opium, taking 52 ml. of the filtered liquid representing 2.5 g. of the original extract.

Tinctura Opii (B.P.). Contains 0.95 to 1.05% *w/v* of morphine, calculated as anhydrous. Assayed by evaporation of 80 ml., trituration with 5 ml. of water, addition of 20 ml. of water and 2 g. calcium hydroxide, followed by addition of water to 86 g., then proceeding as for opium, taking 52 ml. of the filtrate equivalent to 50 ml. of the tincture. Alcohol content, 41 to 46% *v/v*. Tinctura Opii, *U.S.P. XII*, contains 0.95 to 1.05% *w/v* of anhydrous morphine, and has an alcohol content of 17 to 19% *v/v*.

Tinctura Opii Camphorata (B.P.). Contains 0.045 to 0.055% *w/v* of morphine, calculated as anhydrous. Assayed by extraction of the residue, after evaporation, with calcium hydroxide solution, cleaning the solution with ether, addition of ammonium sulphate and extraction with twice the volume of chloroform-alcohol mixture (1 : 1) followed by extraction with chloroform-alcohol mixture (1 : $\frac{1}{2}$); after evaporation of the solvent, the residue is dissolved in N/1 hydrochloric acid and diluted with water to N/10 solution. The brownish colour produced by a 20 ml. portion of the solution on addition of 8 ml. of 1% sodium nitrite solution and 12 ml. of dilute ammonia solution is matched against a standard solution of morphine in N/10 hydrochloric acid. Alcohol content 56 to 60% *v/v*. Tinctura Opii Camphorata, *U.S.P. XII*, yields 0.035 to 0.045% *w/v* of anhydrous morphine; assayed by evaporation with 1/50 volume of N/1 sulphuric acid to low bulk, making just ammoniacal, adding sodium chloride and extraction with alcohol-chloroform (15 : 85); the morphine is subsequently

transferred to 2.5% sodium hydroxide solution saturated with salt, cleaned in hydrochloric acid, salt solution with chloroform, made ammoniacal and extracted with the alcohol-chloroform, evaporated to low bulk, and titrated in alcohol with N/50 sulphuric acid to methyl red indicator.

Tinctura Opii Camphorata Concentrata (*B.P. Add. V*). After dilution and evaporation, it is assayed by the *B.P.* process for Tinctura Opii Camphorata. Contains 0.36 to 0.44% *w/v* of anhydrous morphine.

The Pharmacopœial method for the determination of small amounts of morphine as in camphorated tincture of opium gives high results when applied to other substances owing to the presence of non-morphine chromogenic substances. A quantity of extractive equal to the test solution should be added to the standard before making the comparison. The colorimetric method is then applicable to other preparations containing opium as follows:—

Aromatic Powder of Chalk with Opium. Mix intimately 4 g. of sample with 0.5 g. of slaked lime in a mortar, add water to make a paste, and then transfer quantitatively to a 100 ml. flask with water until about 90 ml. have been used. Shake the mixture occasionally during 30 minutes, make up to volume and filter. Add 0.15 g. of ammonium sulphate to 25 ml. of the filtrate, extract three times with ether, wash the ether with 5 ml. of water and reject the solvent. Extract the aqueous liquid as in the official method for camphorated tincture of opium and make the residue, obtained after evaporation of solvent, up to 25 ml. with N/1 hydrochloric acid before matching.

Powder of Ipecacuanha and Opium. Mix intimately 1 g. of sample with 0.5 g. of slaked lime in a glass mortar. Add water gradually and transfer quantitatively to a 100 ml. graduated flask. Shake the mixture (approximately 90 ml.) frequently during 30 minutes; make up to 100 ml., shake and filter. Extract 25 ml. of filtrate first with ether and then, after adding ammonium sulphate, with alcohol and chloroform as in the Pharmacopœial assay of camphorated tincture of opium. Dissolve the residue in 25 ml. of N/1 hydrochloric acid. Match against standard morphine solution as usual, a convenient amount being 5 ml. of extract and 5 ml. of 0.01% anhydrous morphine solution.

Gall and Opium Ointment. Extract 3.5 to 4 g. of ointment with light petroleum in a Soxhlet apparatus until the residue is free from fat (3 hours). Dry the residue and continue the assay on the total dry powder as given for Dover's powder. Calculate the anhydrous morphine to the original ointment.

Tincture of Chloroform and Morphine. To 10 ml. of tincture in a separator add 5 ml. of water followed by 0.5 ml. of strong solution of ammonia, 15 ml. of alcohol and 15 ml. of chloroform. After shaking and separating, repeat the extraction twice with 8 ml. of alcohol and 15 ml. of chloroform, washing the solvent each time with the same portion of 15 ml. of a 1 : 2 mixture of alcohol and water. After evaporating the solvent, dissolve the residue in 75 ml. of N/1 hydrochloric acid and match 2 ml. against a standard morphine solution.—D. C. Garratt, *Quart. J. Pharm.*, 1937, 466.

OPIUM ALKALOIDS AND DERIVATIVES

Æthylmorphinæ Hydrochloridum (*B.P.C.*).

$C_{19}H_{23}O_3N \cdot HCl \cdot 2H_2O = 385.7$. Loss at 100° , not more than 10%. Ash, not more than 0.1%. A morphine limit equivalent to 0.1% of anhydrous morphine is included by matching against a standard solution the colour produced by 5 ml. of a 2% solution in N/10 hydrochloric acid on the addition of 2 ml. of 1% sodium nitrite solution and 3 ml. of dilute ammonia. **Æthylmorphinæ Hydrochloridum**, *U.S.P. XII*, loses not more than 10% at 100° .

Apomorphinæ Hydrochloridum (B.P.).

$C_{17}H_{17}O_2N \cdot HCl \cdot \frac{1}{2}H_2O = 312.6$. Loss at 100° , not more than 5%. Ash, not more than 0.1%. 0.1 g. shaken with 5 ml. of ether, should produce not more than a faint red colour (due to decomposition products). The *U.S.P. XII* salt complies with the test for decomposition products and is rejected if it produces immediately an emerald-green colour when shaken with 100 parts of water.

Tabellæ Apomorphinæ Hydrochloridi (N.F. VII). Contain 91 to 107% of the labelled amount of apomorphine hydrochloride, including all tolerances. The tablets must be rejected if an emerald green colour is produced immediately when they are dissolved in water in the proportion yielding a 1% solution of apomorphine hydrochloride. Assayed by dissolving a weighed quantity of powdered tablets in water, adding sodium bicarbonate, extracting with ether, washing the ether with water and extracting the aqueous washings with a further portion of ether. The combined ether extracts are extracted with N/20 sulphuric acid and the excess acid titrated with N/50 sodium hydroxide using methyl red indicator.

An official method for the determination of apomorphine in tablets is described in *Methods of Analysis (A.O.A.C., 1940, 587)*. The apomorphine, extracted with peroxide-free ether from a solution made alkaline with sodium bicarbonate, is dissolved in excess standard sulphuric acid and back-titrated with standard sodium hydroxide solution, using methyl red as indicator.

Codeina (B.P.). $C_{18}H_{21}O_3N \cdot H_2O = 317.2$. M.p. of the dried substance, 155° to 156° . Loss at 100° , not more than 6%. Ash, not more than 0.1%. 5 ml. of the 2% w/v solution in N/10 hydrochloric acid, with 2 ml. of 1% sodium nitrite solution and 3 ml. of dilute ammonia solution, produces a yellow colour not deeper than 5 ml. of 0.002% w/v solution of anhydrous morphine in N/10 hydrochloric acid, similarly treated. Codeina, *U.S.P. XII*, loses not more than 6% at 80° ; a test for morphine with ferric chloride and potassium ferricyanide is included; 0.01 g. complies with the test for carbonisable substances.

An official method for the determination of codeine in tablets is described in *Methods of Analysis (A.O.A.C., 1940, 587)*. A solution of the sample in dilute hydrochloric acid is made slightly alkaline with sodium bicarbonate, and extracted with chloroform. The chloroform is removed, the residue dissolved in methyl alcohol and either titrated directly with standard sulphuric acid, or dissolved in excess standard sulphuric acid and back titrated with standard sodium hydroxide, using methyl red as indicator.

Codeinæ Phosphas (B.P.). $C_{18}H_{21}O_3N \cdot H_3PO_4 \cdot H_2O = 415.2$. Loss at 100° , 4 to 7%. Complies with the limit test for morphine. The *U.S.P. XII* substance is the salt with $1\frac{1}{2}$ molecules of water of crystallisation and contains not less than 70% of anhydrous codeine, by extraction from solution with sodium hydroxide by chloroform and titration with N/10 sulphuric acid to methyl red.

Tabellæ Codeinæ Phosphatis (U.S.P. XII). Contain 93 to 107% of the labelled amount of $C_{18}H_{21}O_3N \cdot H_3PO_4 \cdot 1\frac{1}{2}H_2O$, including all tolerances. Assayed by macerating overnight a weighed quantity of powdered tablets with water and N/2 sulphuric acid, filtering and extracting with chloroform the codeine from an aliquot of the filtrate, made alkaline with ammonia solution. After removal of nearly all the chloroform an excess of N/50 sulphuric acid is added, the remainder of the chloroform removed, the solution cooled and titrated with N/50 sodium hydroxide using methyl red indicator.

Codeinæ Sulfas (U.S.P. XII). $(C_{18}H_{21}O_3N)_2 \cdot H_2SO_4 \cdot 5H_2O = 786.5$. Loss at 100° , not more than 12%. Complies with tests for acidity, morphine and carbonisable impurities.

Tabellæ Codeinæ Sulfatis (*U.S.P. XII*). Contain 93 to 107% of the labelled amount of $(C_{18}H_{21}O_2N)_2 \cdot H_2SO_4 \cdot 5H_2O$, including all tolerances. Assayed by the process described for codeine phosphate tablets.

Dihydromorphinoni Hydrochloridum (*U.S.P. XII*). Loss at 100°, not more than 1.5%. Complies with tests for acidity and absence of ammonium salts and codeine.

Tabellæ Dihydromorphinoni Hydrochloridi (*U.S.P. XII*). Contain 90 to 110% of the labelled amount of $C_{17}H_{19}O_3N \cdot HCl$, including all tolerances. Assayed by extraction with chloroform from a weighed quantity of powdered tablets dissolved in saturated sodium bicarbonate solution. After filtration of the chloroform extracts, the chloroform is removed and the alkaloid determined by addition of excess N/50 sulphuric acid and back titration with N/50 sodium hydroxide, using methyl red indicator.

Diamorphinæ Hydrochloridum (*B.P.*).

$C_{21}H_{25}O_5N \cdot HCl \cdot H_2O = 423.7$. Ash, not more than 0.1%. A morphine limit, as indicated by the colour produced in N/10 hydrochloric acid solution with sodium nitrite and ammonia, is included and is equivalent to 1.5%.

An official method for the determination of diamorphine in tablets is described in *Methods of Analysis* (*A.O.A.C.*, 1940, 587). The diamorphine, extracted with chloroform from ammoniacal solution, is dissolved in methyl alcohol and either titrated directly with standard sulphuric acid, or dissolved in excess standard sulphuric acid and back-titrated with standard sodium hydroxide, using methyl red as indicator.

Determination of Diamorphine in Mixture with Procaine. Details are given of a method which depends on hydrolysing the mixture in acid solution, adding excess alcohol and shaking with chloroform and alcohol when only morphine and diethylaminoethanol are extracted and *p*-aminobenzoic acid remains in solution. The former may be separated by the solubility of morphine in lime water.—*Amer. J. Pharm.*, 1938, 362.

Morphina (*B.P.C.*). $C_{17}H_{19}O_3N \cdot H_2O = 303.2$. Loss at 110°, not more than 7%. Ash, not more than 0.1%. Complies with the limit test for other alkaloids in *Morphinæ Hydrochloridum*, using three-quarters the quantity of substance.

Identification. A description and discussion of 21 classified chemical tests for morphine.—*C. C. Fulton, Amer. J. Pharm.*, 1937, 219.

Colorimetric Determination. The reagent is prepared as follows:—Molybdic acid anhydride 14.4 g. is dissolved in 100 ml. of warm N/1 sodium hydroxide and sodium silicate solution is added to bring the SiO_2 content to 0.7 g. 10% hydrochloric acid is then added with stirring until the solution becomes green, and then water to 900 ml. The mixture is placed on a boiling water-bath which is allowed to cool slowly, precipitated silica is filtered off after 24 hours and the volume made up to 1 litre. To 10 ml. of the solution of the sample in 1% hydrochloric acid (containing 0.2 to 10 mg. of morphine) is added 2 ml. of the reagent followed by 5 ml. of 5% ammonium hydroxide solution, and the mixture is diluted to 25 ml. The blue colour produced is matched against that produced by treating similarly a standard solution of morphine in 1% hydrochloric acid. The colour should be compared after standing for 15 to 20 minutes. Reducing agents other than morphine must be removed. Narcotine, papaverine and narceine give only a very faint colour; codeine, thebaine and meconic acid give no reaction although the first two discharge the yellow colour of the reagent.—*C. G. Van Arkel, Pharm. Weekbl.*, 1937, 134.

Determination with 1:2:4-Chlorodinitrobenzene. The following method, a modification of that of Mannich, based on the precipitation of morphine as the 2:4-dinitrophenyl ether, gives accurate results. Dissolve a weighed quantity of morphine salt in alcohol 30% to form a solution not stronger than 0.1% w/v. To 100 ml. of the solution add 10 ml. of strong ammonia solution and 5 ml. of 2% solution of 1:2:4-chlorodinitrobenzene in alcohol 95%. Allow to stand 18 hours, filter through a tared Gooch crucible,

wash with alcohol 30% and finally with ether, dry at 100° and weigh. 1 g. of ppt. is equivalent to 0.632 g. of anhydrous morphine. Insoluble dinitrophenyl ethers are given by other phenolic alkaloids and the method is unsatisfactory for the determination of morphine in opium.—J. R. Nicholls, *Analyst*, 1937, 440.

An official method for the determination of morphine in tablets is described in *Methods of Analysis* (A.O.A.C., 1940, 588). The sample is moistened with water and dissolved in an alkaline salt solution (3% sodium hydroxide in saturated sodium chloride solution). The solution is acidified with hydrochloric acid, and alcohol and a slight excess of ammonia added. The morphine extracted from this solution with chloroform-alcohol (9:1) is dissolved in methyl alcohol, and either titrated directly with standard acid, or dissolved in excess standard acid and back-titrated with standard sodium hydroxide solution. If other alkaloids may be present the initial solution in alkaline salt solution is first extracted with chloroform to remove them. An official method for the determination of morphine in syrups is also described (*ibid.*, 589).

Quantitative methods for the determination of morphine, codeine and diamorphine in tablets are also described.

A colorimetric method for the determination of morphine in hypodermic tablets and injections is described. The method depends upon a modification of Radulescu's colour reaction and tables are given correlating the value of the yellow component of the colour obtained to the morphine content. Details are also given for the determination of diamorphine which may be estimated by the same method after preliminary hydrolysis by refluxing with dilute hydrochloric acid.—N. L. Allport and N. R. Jones, *Quart. J. Pharm.*, 1942, 239.

Spectrum lines characteristic for morphine are obtainable with 1/200 grain.—J. J. Dobbie, *Lancet*, i/1913, 1399.

Bromophenol blue recommended as indicator for titration of morphine. A 1% solution of morphine hydrochloride has a pH of 3.65.—N. Evers, *Yearb. Pharm.*, 1921, 325.

Morphine and other alkaloids in animal excreta. Detection in organs, urine, etc.—*Yearb. Pharm.*, 1922, 12.

The fate of morphine in the animal body, with a method of estimation in body fluids and tissues.—*J. Pharmacol.*, June, 1927, 177.

Distinction from Oxydimorphine. Alkaloid dissolved in a 4% alcoholic solution of *p*-dimethylaminobenzaldehyde and solution warmed on a water-bath. Morphine gives a currant-red and oxydimorphine a green colour.—M. M. Pesetz, *J. Pharm. Chim., Paris*, 1938, 27, 255.

Extraction of Alkaloids from Viscera. The following is a summary of the method recommended.—Freeze the material, mince into a tared casserole while still frozen, add one half its weight of water and warm until homogeneous. Add 50 g. of ammonium sulphate and 2 ml. of glacial acetic acid for every 100 g. of minced material and warm to about 65° until coagulated. Filter in a Buchner funnel, washing the residue with water. If quinine is present, extract the residue with successive portions of water acidified with acetic acid 1%, using 1 to 1½ litres. The washings are collected separately, washed with ether to remove fat and saturate the liquid, rendered alkaline with ammonia and extracted 5 times with 75 ml. portions of ether. The ether is extracted with successive portions of dilute sulphuric acid, the alkaloid transferred to chloroform, and the crude quinine purified by dissolving in ethyl acetate, precipitating as the tartrate, dissolving the latter in water and extracting the base with chloroform after addition of ammonia.

If morphine is present the residue on the filter is extracted with hot saturated ammonium sulphate solution containing 1% of acetic acid until 1½ to 2 litres of filtrate has been collected. The latter is defatted with light petroleum, the aqueous layer rendered ammoniacal, and 100 to 150 ml. of alcohol added carefully to form a small upper layer. The morphine is then extracted with five 100 ml. portions of a chloroform-alcohol mixture (1:1). The extracts are washed twice with small quantities of water and then with three portions of 5% sodium hydroxide, the aqueous and alkaline extracts being acidified, rendered ammoniacal, and extracted with the chloroform-alcohol mixture. The solvent is evaporated and the residue extracted with hot ethyl acetate. The solution is evaporated to dryness, and the residue dissolved in alcohol 30%, and determined by Mannich's method.—C. G. Daubney and L. C. Nickolls, *Analyst*, 1937, 351.

All the acyl compounds of morphine, e.g., diacetylmorphine and benzoylmorphine, are readily decomposed and morphine recovered from them, whereas

the alkyl derivatives, such as codeine and benzylmorphine, are intimate compounds from which the morphine cannot be recovered. The former are strong narcotics like morphine, and the latter only weak narcotics.—D. B. Dott, *Pharm. J.*, ii/1928, 250.

Morphinæ Acetas (B.P.C.). $C_{17}H_{19}O_3N, C_2H_4O_2, 3H_2O = 399.2$. Ash, not more than 0.1%. Complies with the limit test for other alkaloids.

Morphinæ Hydrochloridum (B.P.). $C_{17}H_{19}O_3N.HCl, 3H_2O = 375.7$. Loss at 120° , not more than 12.5% and the dried material is not more than faintly yellow in colour. Ash, not more than 0.1%. Limit of other alkaloids extracted with chloroform from sodium hydroxide solution, not more than 1.5%. Morphinæ Hydrochloridum, *N.F. VII*, should lose not more than 15% at 100° . A test for apomorphine is described; complies with tests for free acid, ammonium salts, meconate, and foreign alkaloids.

GREGORY'S SALT. An impure morphine hydrochloride, being a mixture of double salt of morphine hydrochloride and codeine hydrochloride occurring in the manufacture of morphine.

Assay of Morphine in Morphine Salts. The following process for the assay is recommended by the Sub-Committee on Alkaloidal Assays of the Committee on Pharmaceutical Chemistry:—Transfer about 0.5 g., accurately weighed, to a separator, add 15 ml. of water, 5 ml. of N/1 sodium hydroxide and 10 ml. of chloroform. Shake, allow to separate, and remove the chloroform to another separator. Repeat the extraction with two further quantities each of 10 ml. of chloroform. Wash the mixed chloroform solutions with 10 ml. of N/10 sodium hydroxide, reserve the chloroform, and add the alkaline solution to the first alkaline liquid. Add 20 ml. of alcohol (90%), 40 ml. of a mixture of three volumes of chloroform and one volume of alcohol (90%), and 1 g. of ammonium sulphate. Shake well, allow to separate, and remove the chloroform solution. Repeat the extraction with successive quantities of 30, 20, 20 and 20 ml. of the chloroform-alcohol mixture. Wash each chloroform solution successively with two quantities, each of 5 ml., of water, avoiding vigorous shaking. Filter the chloroform solutions through a plug of cotton-wool, previously moistened with chloroform. Remove the solvent. Add 20 ml. of N/10 sulphuric acid, boil, cool, and titrate the excess of acid with N/10 sodium hydroxide using tincture of cochineal or solution of methyl red as indicator. Each ml. of N/10 sulphuric acid is equivalent to 0.02852 g. of anhydrous morphine. —(*British Pharmacopœia Commission Report*, No. 12, May 1939.) *This method has been included in B.P. Add. IV for the assay of morphine in morphine sulphate.*

Liquor Morphinæ Hydrochloridi (B.P.). Contains 0.95 to 1.05% w/v of morphine hydrochloride. Assayed by extraction from the solution to which alcohol and sodium bicarbonate have been added with chloroform-alcohol (3 : 1), finally drying at 115° and weighing. Alcohol content, 21 to 24% v/v.

Assay. The following process for the assay is recommended by the Sub-Committee on Alkaloidal Assays of the Committee on Pharmaceutical Chemistry:—Transfer 25 ml. to a separator, add 7.5 ml. of alcohol (90%) and 0.5 g. of sodium bicarbonate, and extract the alkaloid by shaking with successive quantities of 40, 30, 20 and 20 ml. of a mixture of three volumes of chloroform and one volume of alcohol (90%). Wash each chloroform solution with the same 5 ml. of water, contained in a second separator. Mix the chloroform solutions, remove the solvent; add 10 ml. of N/10 sulphuric acid, boil, cool, and titrate the excess of acid with N/10 sodium hydroxide, using tincture of cochineal or solution of methyl red as indicator. Each ml. of N/10 sulphuric acid is equivalent to 0.02852 g. of anhydrous morphine. —(*British Pharmacopœia Commission Report*, No. 12, May 1939.)

Morphinæ Sulphas (B.P. Add. IV).
 $(C_{17}H_{19}O_3N)_2.H_2SO_4, 5H_2O = 758.5$. Contains 74.0 to 75.5% of

anhydrous morphine. Determined by the method described under morphine hydrochloride. Other alkaloids, not more than 1.5%. Loss on drying at 120°, not more than 12%. Ash, not more than 0.1%. *Morphinæ Sulfas*, *U.S.P. XII*, allows the same moisture limit, but dries at 130°. Tests for acidity, ammonium salt, meconate and foreign alkaloids are included. On exposure to air, loss of water of crystallisation may result.

Tabellæ Morphinæ Sulfatis (*U.S.P. XII*). Contain 93 to 107% of the labelled amount of $(C_{17}H_{19}O_3N)_2 \cdot H_2SO_4 \cdot 5H_2O$, including all tolerances. Assayed by transferring a weighed quantity of powdered tablets to a separator, using distilled water faintly acidified with hydrochloric acid, making alkaline with ammonia solution, completely extracting with chloroform-alcohol mixture (4:1), washing the combined extracts with water and extracting the aqueous washings with a further portion of chloroform-alcohol mixture. After filtration of the combined extracts, the organic solvent is removed, excess N/50 sulphuric acid added, and the excess acid titrated with N/50 sodium hydroxide, using methyl red indicator. The assay is applicable to hypodermic tablets, but for other tablets a suitable modification of the above method may be necessary.

Tabellæ Morphinæ et Atropinæ Sulfatum (*N.F. VII*). Contain 91 to 109% of the labelled amount of morphine sulphate, including all tolerances. Assayed for morphine sulphate by dissolving as completely as possible in 3% solution of sodium hydroxide saturated with sodium chloride, extracting with ether and rejecting the ether. The solution is neutralised with hydrochloric acid and 0.5 ml. of acid added in excess. Alcohol and a chloroform-alcohol mixture (9:1) are added and the mixture carefully rendered faintly alkaline with ammonia solution, and the alkaloid completely extracted with successive portions of solvent. The filtered and washed chloroform-alcohol solution is evaporated until nearly dry, excess N/20 sulphuric acid added, and the cooled solution titrated with N/50 sodium hydroxide, using methyl red indicator.

Morphinæ Tartras (*B.P.*). $(C_{17}H_{19}O_3N)_2 \cdot C_4H_6O_6 \cdot 3H_2O = 774.4$. Loss at 100°, not more than 7%. Ash, not more than 0.1%. Complies with the limit test for other alkaloids.

Papaveretum (*B.P.C.*). Contains from 47.5 to 52.5% of anhydrous morphine. Assayed by the *B.P.C.* process: extract 1 g. in 20 ml. of water and 5 ml. of N/1 sodium hydroxide with successive portions of 50 ml. and 25 ml. of ether; transfer the aqueous liquid (filtering if necessary) to a 50 ml. flask, and also washings of the ether layers with 2.5 ml. of N/1 sodium hydroxide with 5 ml. of water, and 5 ml. portions of water until 50 ml. has been collected; then transfer to a conical flask, add 5 ml. of alcohol and proceed as the *B.P.* assay for Opium, collecting the morphine on a small filter paper, and adding a correction of 0.025 g. of morphine to the amount indicated by the titration. Opium concentratum, *P.G. VI*, is a mixture of the hydrochlorides of opium alkaloids containing 48 to 50% of morphine.

Papaverina (*B.P.C.*). $C_{20}H_{21}O_4N = 339.2$. M.p., 146° to 147°. Ash, not more than 0.1%. *Papaverinæ Hydrochloridum*, *N.F. VII*, complies with tests for morphine and cryptopine, thebaine, or other inorganic impurities; ash, not more than 0.5%. *Papaverinum hydrochloridum* is official in the *P.G. VI* and in the *Fr. Cx.* 1937.

Thebaine Hydrochloride. Thebainum hydrochloridum is official in the *P. Helv. V*, and contains when dried not less than 99% of $C_{15}H_{21}O_2N \cdot HCl$; it should contain from 2 to 3% of water corresponding to approximately half a molecule of water.

VOLECITHINUM

Lecithin is a mono-aminophosphatide. Phosphatides are complex bodies of more or less fatty nature which can be extracted from tissues by alcohol, ether, etc., and which contain fatty acids, nitrogen and phosphorus. They are of unstable composition.

On hydrolysis lecithin yields stearic acid, glycerophosphoric acid and choline.

Ovolecithinum (B.P.C.) is the lecithin prepared from dried egg-yolk and is required to contain 3.5% of phosphorus.

Lecithins may be derivatives of either stearic, palmitic, or oleic acid, alone or mixed. Ovolecithin is generally assumed to be mainly stearyl, i.e., **choline-distearylglycerophosphate**, and plant lecithin to be mainly an oleic acid body, but the fatty acids are not determined with certainty.

Lecithin Content of Various Substances in percentages—

| | | | |
|----------------------------|------|-----------------------|------|
| Brain | 16.0 | Egg Yolk | 12.0 |
| Heart | 4.5 | Peas | 1.2 |
| Liver | 4.3 | Lupin Seeds | 2.0 |
| Kidneys | 8.5 | Ergot | 1.7 |
| Lung | 1.5 | Yeast (dry) | 2.0 |
| Spinal Cord | 11.0 | Barley | 0.7 |
| Nerve Tissue (dry) | 17.0 | Wheat and Rye | 0.6 |
| Blood Corpuscles | 0.46 | Green Peas | 0.15 |
| Mushrooms | 0.9 | | |

If the cadmium compound of "lecithin" is recrystallised from a mixture of ethyl acetate and 80% alcohol the true lecithin can be freed from kephalin and then liberated from its cadmium compound by means of ammonium carbonate.

Determination of Lecithin in Preparations. Extract 1 g. to 2 g. of a lecithin preparation, or 5 g. to 20 g. of a food stated to contain it, with 96% alcohol—first in the cold and then twice under a reflux condenser. Then extract the insoluble portion with boiling chloroform 2 hours. The combined alcohol and chloroform extractives are evaporated and the residues are digested for 2 hours with 100 ml. of chloroform to separate the lecithin from phosphoric acid, glycerophosphoric acid, etc. To estimate phosphorus pentoxide in the purified extractive, incinerate and oxidise with sulphuric and nitric acids or ignite with magnesium oxide, and bring to weight as pyrophosphate in the usual manner. The factor 11.36 is used to convert the amount found of P_2O_5 into lecithin.

Lecithin is used as an emulsifying agent, especially in the manufacture of chocolate and other confectionery.

C.I.S. Method for Determination of Phosphorus.

The second report of the Commission Internationale des Spécialités includes an agreed method for the determination of phosphorus in organic substances such as lecithin in which the phosphoric acid obtained by treatment with sulphuric and nitric acids is precipitated by means of ammonium molybdate. The washed precipitate, which has approximately the composition $(NH_4)_3PO_4 \cdot 12MoO_3 \cdot 2HNO_3$, is heated with excess of N/2 sodium hydroxide until all ammonia is removed, and the excess of alkali is titrated with N/2 acid using phenolphthalein as indicator. If theoretical equivalents are used the results are slightly low; correct results are obtained by regarding each ml. of N/2 sodium hydroxide as equivalent to 0.560 mg. of P or 1.28 mg. of P_2O_5 .—*Bull. Féd. int. Pharm.*, 1938, 34.

Lipoids are an essential in the food of animals. Among the lipoids there is a series of definite phosphatides, of which the molecule consists of glycerophosphoric acid and a fat acid with a nitrogenous base. Each organ of the body elaborates one or more specific lipoids. Organs or glands which are insufficient or impaired are found to lack lipoids, their power to elaborate them from the materials of the blood being diminished.

OXYGENIUM

Oxygenium (*B.P. Add. I*). $O = 16.000$. Contains not less than 98% *v/v* of O_2 , the residue consisting of argon with a trace of nitrogen, or of hydrogen. Assayed by absorption in one-fifth its volume of alkaline solution of pyrogallol. The turbidity produced in barium hydroxide solution should not exceed that produced by sodium bicarbonate solution equivalent to a limit of CO_2 of approximately 0.0002% *v/v*. Limit tests for halogens and oxidising substances are included. The colour of 100 ml. of water with litmus solution is not changed by 2 litres of gas, by the test for limit of acidity and alkalinity in Nitrogenii Monoxidum—a volume equivalent to 2 litres of the gas at N.T.P. is passed through 100 ml. of boiled water and methyl red solution with 0.2 ml. of N/100 acid; the colour is not more pink than a similar mixture and not more yellow than the water and methyl red with 0.1 ml. of N/100 acid. Oxygenium, *U.S.P. XII*, contains not less than 99% *v/v* of O_2 . The limit of carbon dioxide is one-quarter of that of the *B.P.*; it is assayed by measurement of the residual gas after interaction with ammonium chloride and ammonium hydroxide solution in contact with a copper spiral. The residual gas from approximately 1 litre after absorption in alkaline sodium hydrosulphite solution, shaken with diluted blood, and pyrogallol and tannic acid added shows no pink coloration and no more grey colour than that produced in a blank test using carbon monoxide-free oxygen. Tests for limit of acid, alkalis and halogens are included.

Nitrogenii Monoxidum (*B.P.*). Nitrous oxide drawn from a cylinder in the upright position, contains not less than 93% *v/v* of N_2O . Carbon monoxide limit, determined on the first portion of the gas drawn from an upright cylinder, 50 parts per million *v/v*. Complies with tests for absence of halides and sulphuretted hydrogen, arseniuretted hydrogen and phosphoretted hydrogen, and with limit tests for water vapour, carbon dioxide, uncondensable gases, acidity or alkalinity, reducing substances and oxidising substances. Oxidum Nitrosum, *U.S.P. XII*, complies with tests for carbon dioxide, halogens, reducing substances, and oxidising substances. A test for limit of acids or alkalis is included. The volume of uncondensable gas in the assay process is not more than 5%, indicating not less than 95% *v/v* of N_2O in the gas tested.

PANCREATINUM

Pancreatinum (*B.P.*). Possesses not less than a minimum activity in respect to trypsin, lipase and amylase. To determine the activity due to trypsin, 50 ml. portions of skimmed milk, standardised to protein content, are adjusted to pH 8.0 with N/20 sodium hydroxide using phenol red as external indicator; one portion is boiled and 10 ml. of a 0.5% boiled solution of the

substance added and to the second portion 10 ml. of a 0.5% solution is added, both are heated rapidly and maintained at 38° to 40° for 1½ hours, cooled rapidly and neutralised with N/20 sodium hydroxide to phenolphthalein. Add to each 5 ml. of neutralised solution of formaldehyde and titrate with N/20 sodium hydroxide to phenolphthalein; the boiled mixture requires 4.9 to 5.1 ml. and the other 9.0 to 13 ml. Activity due to lipase is determined by digestion of two 10 ml. portions of a suspension of separated cream in N/10 sodium carbonate containing 0.2% *v/v* of oleic acid and adjusted with acetic acid to pH 8.0, one with 1 ml. of a 1% solution of the pancreatin and the other with 1 ml. of the same solution previously boiled, at 38° to 40°, for 4 hours; after dilution with an equal volume of alcohol, the titration with N/20 sodium hydroxide to phenolphthalein should be not less than 1.0 ml. Amylase is determined by adding to 5 ml. portions of a 0.1% starch mucilage containing 0.5% salt, 0.35, 0.40, 0.45, 0.50, 0.55 and 0.60 ml. of a 0.02% solution of the pancreatin and maintaining at 40° for one hour; after cooling rapidly to 20° and adding one drop of N/10 iodine to each, the portions with addition of 0.5 ml. or more should show no blue colour.

Pancreatinum, *U.S.P. XII*, converts not less than 25 times its weight of starch into soluble carbohydrates and not less than 25 times its weight of casein into proteoses. Pancréatine, *Fr. Cx.* 1937, assayed for proteolytic action by digestion of pork fibrin and titration of the amino-acids produced, yields amino-acids calculated as aminoacetic acid equivalent to not less than 18% of the fibrin; assayed for lipolytic action by titration of the acids produced on reaction with monobutyryne, corresponds to not less than 20% of monobutyryne hydrolysed; and for amylolytic action by titration of the sugars produced on digestion of potato starch.

Assay of Proteolytic Activity. The *B.P.* method is lengthy, the end-point is difficult to observe and the pH should be adjusted before the addition of the formaldehyde. The following assay is based on a formol titration of digested casein.

Casein solution. Dissolve 4 g. of Hammarsten's casein in 90 ml. of water containing 3 ml. of N/1 sodium hydroxide, adjust to pH 8.7, using phenolphthalein as external indicator and dilute to 100 ml.

Neutral standard. To 10 ml. of the *B.P.* phosphate buffer solution at pH 7.0 add one drop of 0.1% solution of neutral red in alcohol (50%).

Alkaline standard. To 10 ml. of the *B.P.* boric acid-potassium chloride-sodium hydroxide buffer solution at pH 8.7 add one drop of 0.1% solution of neutral red in alcohol (50%) and 3 drops of 0.1% solution of phenolphthalein in alcohol (50%).

Preparation of the enzyme solution. Triturate the required weight of the sample with a little chloroform water in a small mortar, wash into a 100 ml. graduated flask and make up to volume with chloroform water. The liquid should not be filtered but should be used as a suspension if insoluble matter is present.

Digestion. Mix 30 ml. of the casein solution and a definite volume of the enzyme solution. Dilute with water to 100 ml., remove 50 ml. as a control and heat the remainder rapidly to 55° keeping at this temperature for 20 minutes. Cool rapidly to laboratory temperature. Add 5 drops of neutral red solution to both liquids and N/10 acid or alkali to both until each colour matches the neutral standard. (This is most easily done by pouring 10 ml. into a test-tube and comparing with the standard in a similar tube.) Add 15 drops of 0.1%

phenolphthalein solution and 10 ml. of formaldehyde solution (B.P.) to both liquids. Titrate with N/10 alkali until the colour matches the alkaline standard. The difference between the two titrations represents the amino-acids formed. The result is preferably expressed as a volume of standard alkali for a definite weight of the enzyme preparation. A convenient method of expression is as the number of ml. of N/1 sodium hydroxide for 1 g. of the sample. The quantity of sample used must be adjusted so that the final titration lies between 4.3 and 4.7 ml.

In the final titration the neutral red changes from pink to yellow before the pink of the phenolphthalein appears. Consequently there is a point in the first colour change at which the tint matches that of the alkaline standard. This must be disregarded. The end-point is reached during the change from yellow to pink.

A reasonable standard for pancreatin would be that 1 g. by the above method should require not less than 15 ml. of N/1 sodium hydroxide in the titration.—N. Evers and W. Smith, *Quart. J. Pharm.*, 1936, 393.

The following assay is recommended by the Sub-Committee on General Organic Chemicals of the Committee on General Chemistry:—Dissolve 4 g., accurately weighed, of purified casein in 90 ml. of water containing 3 ml. of N/1 sodium hydroxide, adjust the pH of the solution to 8.7 and make up the volume to 100 ml. with water. Triturate 0.5 g. of the pancreatin with chloroform water and make up the volume to 100 ml. with chloroform water. Mix 10 ml. of the unfiltered liquid with 30 ml. of the casein solution and make up to 100 ml. with chloroform water. Measure 50 ml. of the mixture into a small flask and heat rapidly to 55°, keeping at this temperature for twenty minutes. Cool rapidly to laboratory temperature. Add 5 drops of solution of neutral red both to the digested liquid and to the 50 ml. which remains undigested. Add to both liquids N/10 sodium hydroxide or N/10 hydrochloric acid until the colour matches the colour produced by mixing 10 ml. of solution of standard pH 7.0 and one drop of solution of neutral red. Add 15 drops of solution of phenolphthalein 0.1% and 10 ml. of solution of formaldehyde to each liquid. Titrate both liquids with N/10 sodium hydroxide until the colour matches the colour produced by mixing 10 ml. of solution of standard pH 8.7, 1 drop of solution of neutral red and 3 drops of solution of phenolphthalein 0.1%. The difference between the two titrations should be not less than 4.5 ml.—(*British Pharmacopœia Commission Report*, No. 14, September 1939.)

Determination of Lipase. A 1—1000 agar solution is adjusted to pH 7, autoclaved at 120° for 15 minutes and filtered while hot, and 20 ml. portions are sterilised in tubes by heating at 115° for 20 minutes. Into a 60 ml. glass-stoppered flask are placed in order glycine 0.6 g., N/1 sodium hydroxide 2 ml., distilled water at 40° 8 ml., and 40 mg. of 1% pancreatin in lactose. The mixture is shaken, 10 ml. of the agar solution (warmed to 40°) is added and 1.5 ml. of tributyrin. The liquid is shaken and maintained at 40° for 2 hours, being shaken for 30 seconds every 5 minutes for the first half-hour and then for 30 seconds every 10 minutes. The pH must remain greater than 8.4. At the end of the 2 hours the action of the enzyme is stopped by adding 1 ml. of 10% sodium metaphosphate and 5 ml. of N/1 sulphuric acid. The liquid is cooled and extracted four times with ether, using 50 ml. for the first extraction and 25 ml. subsequently. The ethereal extract is washed with two quantities of 2 ml. and 1 ml. respectively, of water, diluted with 50 ml. of dehydrated alcohol and 10 ml. of water, and titrated to bromophenol blue with N/10 sodium hydroxide. A blank test is made on an aqueous solution boiled for 2 minutes. The difference in the titration values should be not less than 10 ml.—H. Penau and J. Guilbert, *J. Pharm. Chim., Paris*, i/1937, 5.

Papainum (B.P.C. Supp. IV). Assayed by the B.P.C. '34 process: 0.2 g. in a few ml. of water is washed into 30 ml. of a neutral 4% casein solution, 10 ml. of N/10 sodium carbonate added and diluted to 50 ml.; a 20 ml. portion with 20 ml. of water and 20 ml. of neutral formaldehyde solution is titrated with N/10 hydrochloric acid to phenolphthalein; the remaining 30 ml. of the solution is maintained at 37° for 6 hours and a 20 ml. portion titrated as before; calculated from the difference between the titrations, the amino-acids produced by 1 g. of papain require for

TABLE OF THE COMMON ENZYMES AND FERMENTS

| ENZYME OR FERMENT | CHIEF SOURCE | SUBSTRATE AND PRODUCT(S) |
|--|---|--|
| Amylase and Diastase .. | Human saliva, malt and pancreas. | Hydrolyses starch, forming dextrin and maltose. |
| Catalase (<i>see also Peroxidase</i>) .. | Blood, most animal and plant juices. | Decomposes hydrogen peroxide and other peroxides. |
| Cellulase .. | Grass-eating animals. | Converts cellulose into sugar, as in the case of gramini-voracious feeders. |
| Emulsin .. | Almonds. | Hydrolyses glycosides, e.g., amygdalin. |
| Erepsin | Mucous membrane of small intestine. | Forms simple amino-acids from proteoses and peptones. |
| Fibrin Ferment (<i>see Thrombin</i>) | | |
| Inulase | Inula helenium and squill. | Decomposes inulin, forming fructose. |
| Invertase or Sucrase .. | Intestinal juice and yeast. | Can convert many times its own weight of cane sugar into glucose and fructose. |
| Lactase | Animal body. | Converts lactose into glucose and galactose. |
| Lactic Acid Ferment (organised) | Lactic acid bacteria, <i>q.v.</i> | Converts lactose into lactic acid. |
| Lipase | Pancreatic juice and seeds of plants. | Converts fat into fatty acids and glycerol. |
| Myrosin | Mustard seeds. | Hydrolyses the mustard glycoside in the presence of water. |
| Oxidases (<i>see Catalase and Peroxidase</i>) | | |
| Papain | The juice of <i>Carica Papaya</i> . | Digests proteins in acid or alkaline solution. |
| Pepsin | Stomach, e.g., pigs. | Converts proteins into meta-protein, proteoses and peptone, in acid solution only. |
| Peroxidase .. | Blood, milk and many plant tissues, e.g., potato and fungi. | Oxidiser. Sets free oxygen from H_2O_2 . When an organic peroxide is in the plant tissue the system is called oxidase. |
| Perhydridase .. | Ditto. | Reducing agent. |
| Ptyalin | Saliva of the mouth. | Converts cooked starch into dextrin and maltose. |
| Rennin or Chymosin .. | Stomach of sucking animals, e.g., calf. | Coagulates the casein in milk, effecting clotting. |
| Steapsin, or Lipolytic Ferment | <i>See Lipase.</i> | |
| Thrombin .. | Blood, after it is shed, in the presence of Ca salts. | Coagulates fibrinogen into fibrin, forming the clot. |
| Trypsin | Pancreas. | Converts proteins into amino-acids and a polypeptide in dilute alkali. |
| Urease | The soya bean and in urine, especially in catarrh of bladder. | Converts urea into ammonium carbonate. |
| Zymase | Yeast. | Converts sugars into alcohol and CO_2 . |

neutralisation not less than 20 ml. of N/10 sodium carbonate. Ash limit, 10%. The *Fr. Cx.* 1937 estimates the proteolytic action of papain by digesting a mixture of 0.015 g. with 2.5 g. dried pork fibrin and 50 ml. water at 70° for 2 hours; after filtering 10 ml. of the filtrate does not show any change with 20 drops of nitric acid; 10 ml. evaporated to dryness weighs not less than 0.3 g. and in a 2 decimetre tube has a rotation of not less than -2.4° ; loss at 102°, not more than 12%; ash not more than 12%.

Pepsinum (B.P.). Dissolves not less than 2500 times its weight of coagulated egg albumen. Assayed by digestion, at 40° to 41° for 6 hours, of a solution of pepsin and salt in water acidified with hydrochloric acid with a trituration of freshly prepared egg albumen in acidified water, when no granules should be visible and the liquid not more than faintly opalescent. Pepsinum, *N.F. VII*, should digest 3000 to 3500 times its weight of freshly coagulated and disintegrated egg albumen. The digestion is conducted at 52° during 2½ hours, and the volume of undigested albumen measured with comparison to a Reference Pepsin solution. The *Fr. Cx.* 1937 requires that pepsin shall convert 50 times its weight of dried fibrin, corresponding to a proteolytic value of 200:—digest 1% pepsin solution, 5 ml., N/1 hydrochloric acid; 4.3 ml., fibrin, 2.5 g., water, 60 ml., for 6 hours at 50°. The pH of the liquid should then be 3.4. Test the filtrate with nitric acid. Pepsine Amylacée and Pepsine Lactosée correspond to the above test using a solution containing 2.5 g. in 100 ml., which corresponds to a proteolytic value of 80. Pepsinum, *P. Helv. V*, is standardised against a known weight of casein and diluted if necessary with lactose; the moisture should not exceed 5% and the ash 3.5%.

The *B.P.* assay process, retained from the *B.P.* '14, is unsatisfactory, and should never be used except as an empirical limit test, since it does not measure the amount of egg albumen dissolved. Pepsin is only manufactured in two strengths, and dilution of the lower strength should not have been sanctioned.—*Pharm. J.*, ii/1932, 146.

After consideration of a number of methods proposed for the assay of pepsin, that described by Cole (*Practical Physiological Chemistry*, 9th Edn., p. 228) based on the clotting of calcified milk, is recommended.—A. C. Munro and R. Seifert, *Pharm. J.*, i/1933, 482.

For a method depending on the digestion of soluble casein see under *Ventriculus Desiccatus*, p. 204.

As the result of the examination of 13 different samples of pepsin, it is concluded that commercial pepsins on the English market to-day are of good quality. A discussion and comparison of the *B.P.*, *U.S.P.*, *P.G.*, and edestin methods of assay are given, and figures are deduced showing the comparative severity of the tests. Suggestions are made for an improved official assay process. The greatest obstacles in the way of obtaining comparable results in the testing of pepsins is shown to be the variability of the substrate. In the case of egg-white, two methods of overcoming this difficulty are:—(a) the setting-up of a standard pepsin with which other pepsins are to be compared in tests using the same egg-white for tests and standard, or preferably (b) the use of a method of averaging the results of several tests.—K. Bullock, *Quart. J. Pharm.*, 1935, 13.

Carminic Fibrin; prepared by washing blood fibrin with ammoniacal solution of carmine, is a dark-coloured mass, easily crumbled, which yields no colour to water or 0.1% hydrochloric acid until the fibrin contained in it has been dissolved by a ferment; hence its use as a simple quantitative test for pepsin by noting the time required to give a pink colour equal to that of a standard or control.

Pepsin Solution. Loss of activity on storage. A mixture containing pepsin in acidified chloroform water showed a reduction of 40% in activity after a fortnight. A concentrated solution, containing 4 g. per fluid ounce of chloroform water but no acid, was stable.—A. C. Munro and R. Seifert, *Pharm. J.*, i/1933, 432.

Peptonum. The *Fr. Cx.* 1937 requires Peptone Pancréatique to contain about 14.5% of total nitrogen. 0.5 g. in 50 ml. water titrated with N/10 soda to phenolphthalein, should require about 8 ml. and on the addition of 10 ml. of neutralised formaldehyde solution and further titration should require about 20 ml. for neutralisation of the amino-acids; the ratio of nitrogen of amino-acids to total nitrogen should be greater than 0.2. Peptone Pepsique, *Fr. Cx.* 1937, should contain about 14.5% of total nitrogen and the ratio of amino-acid nitrogen to total nitrogen should be less than 0.2.

Meat Extract.

Extractum Carnis (N.F. VII). Total solids, not less than 75%; ash, not more than 30% of the total solids; sodium chloride, determined on the ash, not more than 10% of the solids; alcohol-insoluble solids, dried at 110°, not more than 10% of the solids. Nitrogen content of alcohol-soluble solids determined on the alcoholic filtrate from the insoluble solids by the Kjeldahl method, not less than 6%; NH_3 , not more than 0.35% of the total solids determined by distillation of 100 ml. of the solution of the extract with 5 g. barium carbonate and 100 ml. water.

Methods for the determination of constituents of meat extracts are described in *Methods of Analysis (A.O.A.C., 1940, 384 et seq.)*. The tentative method for ammonia consists of aspirating a series of tubes so that the air passing through a sulphuric acid (1+9) wash bottle passes through a tube containing the extract with 1 ml. of saturated potassium oxalate solution, a few drops of kerosene and sufficient sodium carbonate solution to make just alkaline, and into a titration tube containing standard acid.

Tentative Methods for Amino Nitrogen.

Van Slyke Method: The filtrate from a water-extract boiled with 1 ml. of N/1 acetic acid is shaken with glacial acetic acid and sodium nitrite solution (3%), the nitrous oxide and nitrogen formed shaken with potassium permanganate, and potash solution and the unabsorbed nitrogen measured, correcting for a blank determination on the reagents.

Sorensen Method: To 20 ml. of the filtrate from which the coagulable and insoluble nitrogen have been removed as in the above process and neutralised to phenolphthalein, 10 ml. of neutralised formaldehyde is added and the mixture titrated with N/5 barium hydroxide, adding a slight excess and back titrating with N/5 hydrochloric acid. A blank determination is made and the nitrogen present as free ammonia subtracted.

Seriparium (B.P.C.). Rennet (rennin) should comply with the following test:—Mix 0.1 g. with 50 ml. of water, allow to stand for 15 minutes and add 1 ml. to 50 ml. of milk, of acidity to phenolphthalein equivalent to 0.14 to 0.15% of lactic acid, warmed to 43° in a beaker about 12 cm. high and 5 cm. in diameter; stir slowly for 10 seconds and maintain at 43°; thickening commences within 10 minutes, and a firm curd is produced within an additional 30 seconds, indicating coagulation of not less than 25,000 times its weight of fresh cows' milk. Renninum, *N.F. VII*, is standardised by addition of 1 ml. of rennin solution (0.2%) to 50 ml. of well-mixed cows' milk at 43°, stirring for 10 seconds and timing the period taken for thickening as shown by a convex surface when the vessel (12 cm. high and 4.5 cm. diameter) is tipped at an angle of 45°; it coagulates in 90 to 110% of the time

taken by a reference rennin solution. The reference solution is prepared from a powdered rennin, the stability and standards of which have been definitely established over a number of years, coagulating approximately and not less than 25,000 times its weight of fresh cows' milk.

Rennin differs considerably from pepsin. It is a decomposition product of protein, of acid albumin type, not precipitated by boiling the solution (*cf.* pepsin). It dialyses through parchment but is hydrolysed in the process (the main bulk in the case of pepsin its not dialysed). Rennin is precipitated on saturating the liquid with sodium chloride. Proteolytic activity does not seem to be a part of the true physiological characteristics of rennin.—*J. Amer. chem. Soc.*, 1923, 249, per *Chem. & Drugg.*, i/1923, 437.

PARAFFINUM

Paraffinum Durum (B.P.). M.p., 50° to 60°. Ash, not more than 0.05%. Paraffinum, *N.F. VII*, melts between 47° and 65°, and complies with a test for carbonisable substances.

Paraffinum Liquidum (B.P. Add. I). Sp. gr., 0.880 to 0.895. Kinematic viscosity, not less than 64 centistokes at 37.8°. In the sulphuric acid test, the acid layer is separated from the heated mixture and when viewed in a cell in a colorimeter is not deeper in colour, either in respect to red or yellow components, than the combination of colour glasses prescribed. Petrolatum Liquidum, *U.S.P. XII*, has a kinematic viscosity of not less than 38.1 centistokes at 37.8°. Sp. gr. at 25°, 0.860 to 0.905. Huile de Vaseline Fluide, *Fr. Cx.* 1937, should have a viscosity of 10 to 16 centistokes; water and alcohol boiled with it should be neutral to bromothymol blue.

The important test for impurities with sulphuric acid is now carried out with nitrogen-free sulphuric acid, the strength of which is 96%. Probably most chemists have used 98% acid for this test, and the reduction in strength makes a very great difference in the amount of impurity which the test will detect, and the change may lead to a lowering in the standard of purity. Probably the taste is the most important point about the oil, and it is unfortunate that no standard for this quality can be laid down.—*N. Evers, Pharm. J.*, i/1933, 195.

Deterioration of Liquid Paraffin during Storage.

Some samples of liquid paraffin develop objectionable odours on standing. The following test for the formation of peroxides during autoclaving indicates the degree of deterioration to be expected on long storage. Place 100 ml. of the sample in a wide-mouthed 8 oz. screw-capped jar (with lining removed from cap), loosely replace cap and autoclave at 20 lb. pressure for one hour. To 10 ml. of cooled autoclaved oil in a test-tube add 5 ml. of testing solution (*infra*); replace air in the test-tube by carbon dioxide or nitrogen, cork tightly and shake for 30 seconds. A pink or red colour in the aqueous layer denotes the presence of peroxides and indicates that the sample is liable to deteriorate on storage. The testing solution is made as follows:—*Solution A*.—10 g. of ferrous sulphate is dissolved in 500 ml. of water to which has been added 10 ml. of sulphuric acid and 1 g. of potassium thiocyanate. When solution is complete 1000 ml. of commercial acetone is added and the mixture refluxed in the presence of bright iron wire, a stream of carbon dioxide or nitrogen being simultaneously passed through it. The colourless solution is stored over bright iron wire in a bottle in which the air is replaced by an inert gas. *Solution B* is a 2% w/v solution of potassium thiocyanate. For use mix 3 volumes of solution A with 1 volume of solution B and store out of contact with air in a bottle containing bright iron wire. It is ready for use when it is colourless or has a slate-grey cast.—*P. L. Burrin, A. G. Worton, and F. E. Bibbins, J. Amer. pharm. Ass.*, 1936, 27.

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Paraffinum Liquidum Leve (*B.P. Add. IV*). Sp. gr., 0.835 to 0.875. Kinematic viscosity, not greater than 33.1 centistokes at 37.8°. Limits of sulphur compounds and of acidity are included. *Petrolatum Liquidum Leve, U.S.P. XII*, has a kinematic viscosity of not more than 37 centistokes at 37.8°. Sp. gr., 0.828 to 0.880.

Paraffinum Molle Album (*B.P.*). n_{D60}° , 1.453 to 1.460; m.p., 40° to 46°. Volatilises without acrid odour and complies with tests for acidity, fixed oils, soaps and resins. The *B.P. Add. VI* states that yellow soft paraffin may be used or supplied in place of white soft paraffin. *Petrolatum Album, U.S.P. XII*, complies with the tests for *Petrolatum, U.S.P. XII*.

Paraffinum Molle Flavum (*B.P.*). n_{D60}° , 1.460 to 1.474; m.p., 38° to 46°; ash limit, 0.05%. Volatilises without acrid odour and complies with tests for acidity, fixed oils, soaps and resin. *Petrolatum, U.S.P. XII*, melts between 38° and 60°; sp. gr. at 60°, 0.815 to 0.865; ash, not more than 0.05%.

The lower limit of melting-point of yellow soft paraffin has been lowered from 42° to 38°, and the latter now includes Vaseline.—N. Evers, *Pharm. J.*, i/1933, 195.

A simple test is prepared to distinguish between "natural" and "artificial" soft paraffin. The test consists in determining the deposition temperature or solution temperature of a solution of the soft paraffin in a mixture of glacial acetic acid and carbon tetrachloride. The volumes of crystalline deposit, if any, and of the upper oily layer which separates are also determined. Freedom from adulteration with liquid paraffin and hard paraffin is indicated by (1) a high solution temperature, (2) a low volume of crystalline deposit and (3) a considerable volume of separated upper oily layer.—H. Brindle, *Quart. J. Pharm.*, 1939, 361.

Iodine Value. A modification of the Wijs method giving fairly constant results is described. The *B.P.* method is unsuitable because soft paraffins are largely insoluble at ordinary temperatures in the reagents used, and the pyridine sulphate bromide method has no special advantages. Using the method described, 18 samples of yellow soft paraffin gave an average iodine value of 9.6, and 19 of white soft paraffin gave an average value of 5.1. As a rule there was a close relationship between the iodine values of soft paraffins and their tendency to become overheated in the preparation of bleach ointment, the higher the value, the greater the tendency towards over-heating.—H. Brindle, *Analyst*, 1940, 409.

Petroleum Leve (*B.P.C.*). Not less than 95% distils between 40° and 60°, the sp. gr. of which is 0.620 to 0.700. Residue on evaporation, not more than 0.002% *w/v*. *Benzinum Purificatum, U.S.P. XII*, has a sp. gr. at 25° of 0.634 to 0.660. Distils completely between 35° and 80°. Residue on evaporation not above 40°, not more than 0.002%.

Cera Alba (*B.P.*). Acid value, 18 to 24; the wax complies with the other constants and tests for *Cera Flava*. *Cera Alba, U.S.P. XII*, has an acid value of 17 to 24, an ester value of 72 to 79, and responds to the other tests for *Cera Flava, U.S.P. XII*.

Cera Flava (*B.P. Add. I*). Sp. gr., 0.958 to 0.970; m.p., 62° to 64°; n_{D60}° , 1.4380 to 1.4420. Acid value, by titration in absolute alcohol with N/2 alcoholic potassium hydroxide, 17 to 23. Ester value, 70 to 80. Ratio number of 3.3 to 4.2. *Cera*

Flava, *U.S.P. XII*, has an acid number of 18 to 24, and an ester number of 72 to 77.

Cetaceum (*B.P.C.*). M.p., 42° to 50°; $n_{D, 80^{\circ}}$, about 1.4330. Acid value, not more than 1.0. Saponification value, 120 to 136. Iodine value, 3 to 4.4. Cetaceum, *U.S.P. XII*, has a sp. gr. of about 0.94 at 25°. M.p., 42° to 50°. Tests for paraffin and free acids and for stearic acid are included.

PELLETIERINA

Pelletierinæ Tannas (*B.P.*). Residue on ignition, not more than 0.1%. An acidified solution yields no precipitate with platinic chloride solution. Pelletierinæ Tannas, *U.S.P. XII*, should yield not less than 20% of residue by extraction with chloroform from alkaline solution, evaporation with 0.1 ml. of hydrochloric acid, and drying for one hour at 60°.

Granati Radicis Cortex (*B.P.C.*). Contains not more than 2% of wood or other foreign organic matter. Écorce de Grenadier, *Fr. Cx.* 1937, contains not less than 0.25% of alkaloids by the following process:—Macerate 15 g. of the dried drug for 2 hours with 5 g. calcined magnesia and 10 ml. water, and then reflux with 150 ml. of chloroform for 1 hour. After readjustment, evaporate 100 ml. of the filtered chloroform to 40 ml., transfer to a separator with 40 ml. of ether and extract with 10 ml. of N/10 hydrochloric acid and 20 ml. water, followed by 2 portions of 20 ml. water; back titrate the acid liquids with N/10 potash using methyl red indicator; 1 ml. N/10 acid = 0.01475 g. alkaloid. Cortex Granati, *P.G. VI*, should yield not less than 0.4% of alkaloid. Cortex Granati, *P. Helv. V*, is standardised to yield not less than 0.5% of alkaloid.

It is shown that Ewer's Method (*Arch. Pharm.*, 1899, 237, 53, 56) for the estimation of the alkaloids of pomegranate bark gives fairly accurate results, that it is possible to remove the inactive pseudo-pelletierine from the total alkaloids and that commercial "pelletierine" salts are apt to be low in "pelletierine" content and therefore of doubtful value as tænicides.—H. J. A. Goodson, *Quart. J. Pharm.*, 1940, 56.

PHENACETINUM

Phenacetinum (*B.P.*). $C_{10}H_{13}O_2N = 179.1$. M.p., 134° to 136°. Ash, not more than 0.05%. Complies with tests for neutrality, readily carbonisable substances, acetanilide and *p*-phenetidine. Acetophenetidinum, *U.S.P. XII*, melts between 134° and 136°. Ash, not more than 0.05%.

Tabellæ Acetophenetidini (*U.S.P. XII*). Contain 94 to 106% of the labelled amount of $C_{10}H_{13}NO_2$, including all tolerances. Assayed by extraction of a weighed quantity of powdered tablets with chloroform after preliminary extraction with light petroleum, removal of the chloroform and weighing the residue, dried at 60°.

Tentative Method for the Determination of Phenacetin and Acetanilide in Mixtures. *Methods of Analysis* (A.O.A.C., 1940, 560). 0.2 g. of the mixture is heated with 2 ml. glacial acetic acid and diluted with 40 ml. of water at 70°. To the clear liquid 25 ml. of iodine solution (3% of purified iodine and 4% of potassium iodide, standardised to thiosulphate) and 3 ml. of hydrochloric acid are added. When crystallisation commences the mixture is allowed to cool and stand, and the crystals of periodide filtered off. The excess iodine in the filtrate is titrated with a standard thiosulphate solution or the iodine removed from the crystals with a little sodium thiosulphate and the phenacetin extracted with chloroform. Acetanilide is determined in the filtrate from the phenacetin periodide, decolourised with thiosulphate; a slight excess of sodium bicarbonate followed by 1 or 2 drops of acetic anhydride are added and the liquid extracted with chloroform. The chloroform is evaporated to low bulk and digested on a steam-bath with sulphuric acid (1+9), hydrochloric acid is added and the liquid titrated with N/10 bromine. Details for the removal of caffeine and antipyrine, if present, are given.

Tentative Method for the Determination of Phenacetin and Caffeine. *Methods of Analysis* (A.O.A.C., 1940). For caffeine the product obtained by the gross separation of the caffeine-phenacetin mixture in chloroform is treated with 10 ml. of sulphuric acid (1+9) and heated on the water-bath until the volume is reduced to 5 ml. This is treated with 10 ml. of water and the volume again reduced to 5 ml.; a further 10 ml. of water is added, the volume reduced to 8 to 10 ml. and the procedure repeated until there is no further odour of acetic acid. (Care must be taken that phenetidin sulphate is not formed by excessive concentration of the solution). The cooled mixture is diluted to about 20 ml. and the caffeine extracted with chloroform. For phenacetin the solution of phenetidin sulphate is made slightly alkaline with sodium bicarbonate, 50 ml. of chloroform and 5 drops of acetic anhydride for every 0.1 g. of phenacetin believed to be present are added, the mixture shaken vigorously and the chloroform washed with water. The extraction is repeated with 2 further portions of chloroform, the chloroform evaporated, excess acetic anhydride removed by repeated evaporations with 1 ml. of chloroform, and the residue of reformed phenacetin weighed after drying in air or in a vacuum desiccator over lime.

Method for the Determination of Phenacetin and Caffeine in Tablets. The second report of the Commission Internationale des Spécialités includes an agreed method for the determination of phenacetin and caffeine in tablets in which the phenacetin and caffeine are extracted with chloroform and the phenacetin hydrolysed by dilute sulphuric acid to phenetidin which remains in solution as the sulphate. The solution is diluted with water and the caffeine extracted with chloroform, and the residue on evaporation of the chloroform is dried and weighed. The aqueous solution of phenetidin sulphate is treated with a slight excess of sodium bicarbonate and chloroform and acetic anhydride added. After shaking, the chloroform layer is drawn off, washed, and filtered, and the chloroformic extraction is repeated twice. The mixed chloroform solutions are then evaporated and the residue of phenacetin dried and weighed.—*Bull. Féd. int. Pharm.*, 1938, 85.

Tabellæ Acetophenetidini et Phenylis Salicylatis (N.F. VII). Contain 90 to 110% of the labelled amounts of acetophenetidin and phenyl salicylate, including all tolerances. Assayed for phenacetin by extracting with chloroform, removing the chloroform, hydrolysing the salol by heating with 2.5% sodium hydroxide for 15 minutes over a steam-bath, extracting the phenacetin from the cooled alkaline liquid with chloroform, filtering the combined chloroform extracts, removing the chloroform, drying and weighing. Assayed for salol by treating the alkaline liquid from the preceding assay as described under salol tablets.

PHENAZONUM

Phenazonum (B.P.). $C_{11}H_{12}ON_2 = 188.1$. M.p., 111° to 113°. Ash, not more than 0.1%. Antipyrina, U.S.P. XII, leaves not more than 0.15% of ash. Phényldiméthylpyrazolone, Fr. Cx. 1937, contains not less than 99% of the pure substance:—Dissolve

0.1 g. in 10 ml. water, add 1 g. potassium carbonate and 20 ml. N/10 iodine; after 1 hour add 1 ml. acetic acid then 10 ml. of chloroform and back titrate with N/10 sodium thiosulphate; 1 ml. N/10 iodine = 0.0094 g. phenazone.

Determination. It may be precipitated as $(C_{11}H_{11}N_3O)_2 \cdot H_2Fe(CN)_6$ from an acid solution by means of potassium ferrocyanide. A weighed quantity of the sample, containing 0.2 to 0.3 g. of phenazone, dissolved in 30 ml. of 0.8 N HCl is treated with 20 ml. of M/2 potassium ferrocyanide added slowly with stirring. Allow to stand for 30 minutes, collect in a Gooch crucible, wash with saturated aqueous phenazone hydroferrocyanide and dry at 105° to 110° . Add a correction of 0.005 g. for solubility. The precipitate contains 63.53% of phenazone. A volumetric method for determining the precipitate is also described. Amidopyrine does not interfere with either result.—I. M. Kolthoff, *J. Amer. pharm. Ass.*, 1933, 947.

0.2 g. of the antipyrine and 2 g. of sodium acetate are dissolved in 20 ml. of water in a stoppered flask and 30 ml. of N/10 iodine solution added. The flask and contents are allowed to stand with occasional shaking for 20 minutes. The precipitate is then dissolved by adding 10 ml. of chloroform and shaking, and the excess of iodine titrated with N/10 sodium thiosulphate solution. The amount of iodine reacting with the reagents is determined by repeating the process, omitting the antipyrine, or preferably by using only 10 ml. of N/10 iodine for the blank determination. An allowance is made for the iodine which reacts with the reagents.—H. Brindle, *Quart. J. Pharm.*, 1934, 453.

An official method for the determination of mixtures of phenazone and caffeine is described in *Methods of Analysis (A.O.A.C.)*, 1940, 573).

To 10 to 15 ml. of solution containing about 0.1 to 0.2 g. of phenazone add sufficient hydrochloric acid to make the content of HCl 5%. Add 0.5 to 1.0 g. of potassium bromide and 1 drop of a 0.2% alcoholic solution of *p*-ethoxy-chrysoidine and titrate with N/10 or N/100 potassium bromate until the red colour disappears. Each ml. of N/100 potassium bromate is equivalent to 0.94055 g. of phenazone. Caffeine sodium benzoate, caffeine citrate, phenacetin, acetylsalicylic acid and glycerol do not interfere.—E. Schulek and J. Kovács, per *Chem. Abstr.*, 1940, 34, 7063.

Phenazoni Salicylas (B.P.C.). $C_{11}H_{12}ON_3 \cdot C_7H_6O_3 = 326.2$. Contains not less than 57% of phenazone, and not less than 42% of salicylic acid. M.p., 91° to 92° . Ash limit, 0.1%. Assayed for phenazone by extraction with chloroform from sodium hydroxide; the residue on evaporation responds to the identity tests for Phenazonum. Salicylic acid content is determined by titration in 60% alcohol solution with N/10 sodium hydroxide to phenol red.

Antipyrinum salicylicum, *P. Helv. V*, contains 42.0 to 42.3% of salicylic acid when a solution of 1 g. in 20 ml. of alcohol and 30 ml. of water is titrated with N/10 sodium hydroxide, using phenolphthalein as indicator.

Amidopyrina (B.P.). $C_{13}H_{17}ON_3 = 231.2$. M.p., 107° to 109° . Ash limit, 0.1%. Aminopyrina, *U.S.P. XII*, complies with a test for antipyrine, in which 0.1 g. should develop no more colour when heated to boiling with 0.1 g. of vanillin, 5 ml. of water and 2 ml. of sulphuric acid than a control omitting the aminopyrine.

An official method for the determination of amidopyrine is described in *Methods of Analysis (A.O.A.C.)*, 1940, 573). The amidopyrine, extracted with chloroform from a solution made alkaline with ammonia or sodium hydroxide solution, is dried and weighed. Melting point and identity tests are applied to the residue.

Detection of Phenazone in presence of Amidopyrine. Use is made of the reaction between phenazone and ammoniated mercury in warm aqueous solution: after cooling, potassium iodide is added to the filtrate and the solution titrated with 0.1N Iodine. Amidopyrin does not react under these conditions.—F. Monforte, per *Brit. chem. Phys. Abstr. B.*, 1938, 977.

Tabellæ Amidopyrinæ (N.F. VII). Contain 92.5 to 107.5% of the labelled amount of amidopyrine, including all tolerances. Assayed by extracting a weighed quantity of powdered tablets with N/1 hydrochloric acid, filtering and rendering alkaline with ammonia solution an aliquot of the filtrate, extracting with chloroform, washing the combined chloroform extracts with water, extracting the washings with a further portion of chloroform, filtering the combined chloroform extracts, removing the chloroform, drying and weighing the residue.

PHENOL

Phenol (B.P.). $C_6H_5O = 94.05$. Contains not less than 98% of the pure substance. Residue on volatilisation on a water-bath, not more than 0.05%. Assayed for bromine absorption by a modified Koppeschaar process (*vide infra*).

The following *British Standard Specifications* have been prepared by the British Standards Institution for different grades of phenol and carbolic acids. Each specification contains descriptions in detail of the tests which are applied and descriptions of the apparatus used.

B.S.S. No. 523—1933 relates to crystalline phenol and liquefied phenol or solutions of phenol.

B.S.S. No. 515A—1933 and *No. 515B—1933* refer to crude carbolic acids, 60's and 45's. Crude carbolic acid 60's contains not more than 15% of water, has a gravity not lower than 1.055 and shows not more than 6% of residue on distillation, whilst crude carbolic acid 45's contains not more than 12% of water, has a gravity not lower than 1.050 and shows also not more than 6% of residue on distillation.

Quantitative Estimation of Phenol, Koppeschaar Process. This depends upon converting it into tribromophenol, $C_6H_2Br_3OH$:—

Dissolve phenol, 1.5 g., in water sufficient to make 1000 ml. Place 25 ml. of the solution in a 200 ml. stoppered bottle, add 30 ml. of N/10 bromine solution (*Koppeschaar's Solution*) and shake repeatedly for half an hour; allow to stand 15 minutes, then add 5 ml. of 10% potassium iodide solution, shake well, add 1 ml. of chloroform and titrate excess of iodine with N/10 thiosulphate. Subtract the number of ml. required from thirty; the remainder equals the number of ml. of N/10 bromine used up. This multiplied by 4 gives the percentage of absolute phenol (i.e. 1 ml. N/10 Br = 0.001568 g. of phenol).

Koppeschaar's Bromine Solution is made as follows:—

Dissolve potassium bromate, 3.2 g., and potassium bromide, 50 g., in water, 900 ml. To standardise, place 20 ml. in a 250 ml. bottle with 75 ml. of water and 5 ml. of hydrochloric acid. Shake a few times, quickly introduce 5 ml. of 20% potassium iodide solution and titrate the iodine set free with N/10 sodium thiosulphate. Dilute the bromine solution so that equal volumes of it and the N/10 thiosulphate exactly correspond in the conditions of the test.—*cf. U.S.P. XII*, p. 749, and *B.P.*, p. 513.

Phenol Liquefactum (B.P.). Contains 78.5 to 81.5% *w/w* of phenol. The *B.P. Add. I* directs that Phenol Liquefactum be completely liquefied before use if congealed or if crystals have deposited through storing below 4°. The *U.S.P. XII* preparation contains not less than 88% *w/w* of C_6H_5OH ; b.p., not above 182°.

Trochiscus Phenolis. Assay. For the assay of phenol in preparations containing ingredients which react with bromine a known quantity of the preparation containing about 0.15 g. of phenol is placed in a flask with 125 ml. of water, acidified with hydrochloric acid, and 25 g. of anhydrous calcium chloride added. The mixture is distilled until about 100 ml. of distillate is collected. The volume of distillate is adjusted exactly to 100 ml. and the phenol determined in 30 to 40 ml. of the solution by the *B.P.* method. In the case of phenol lozenges, allowance should be made for loss of phenol during manufacture. The standard should be set on the percentage of phenol present and

not on the amount in each lozenge. The lozenges should be required to weigh not less than 1.10 and not more than 1.22 g., and contain not less than 1.75% of C_6H_5OH .—C. E. Corfield and L. M. Mundy, *Quart. J. Pharm.*, 1932, 504.

The following is an alternative process:—The lozenges are first weighed, and crushed. A convenient weight of the crushed material, usually about 3 to 4 g., is dissolved in water, and the solution adjusted to a volume of 100 ml. 25 ml. of this solution is placed in a 300 ml. flask, and diluted with water to a volume of 150 ml. The flask is fitted to a condenser, and the contents distilled until only about 5 ml. remain in the flask. An asbestos ring should be used to prevent overheating. Distillation may be rapid at first, but towards the end, caution is required to prevent overheating and possible charring of the residue. The distillate is collected in a 300 ml. stoppered bottle, and the temperature brought to about 20°. 30 ml. of N/10 iodine and 30 ml. of N/10 sodium carbonate are added, and the mixture allowed to stand for 5 minutes. The mixture is then acidified by the addition of 5 ml. of dilute sulphuric acid, and the excess iodine is titrated with N/10 sodium thiosulphate. Each millilitre of N/10 iodine is equivalent to 0.001567 g. of phenol.—C. A. Hill and A. D. Powell, *Quart. J. Pharm.*, 1934, 535.

Unguentum Phenolis. Assay. The phenol may be separated from the base in a form suitable for titration with bromine either by distillation from acid solution or by solution in N/1 sodium hydroxide and separation of the phenol solution by means of calcium chloride.—E. M. Smelt, *Quart. J. Pharm.*, 1932, 509.

Dissolve 0.5 g. in about 10 ml. of light petroleum, and extract with four 10 ml. portions of a solution of 7 ml. of hydrochloric acid and 33 ml. of water, shaking for two minutes on each occasion. Collect the extracts in a Wijs iodine flask, add 20 ml. of N/10 bromate-bromide, and complete the analysis in the usual way. Two analyses gave results of 99.6% and 100.8% of the theoretical amount of phenol.—R. M. Savage, *Quart. J. Pharm.*, 1939, 420.

Unguentum Phenolis, U.S.P. XII. is determined by titration, with N/10 bromine, of the liquid obtained by steam distillation. C_6H_5OH content, 1.8 to 2.2%.

Betanaphthol (B.P.). $C_{10}H_8O = 144.1$. M.p., 120° to 122°. Ash, not more than 0.05%. No violet colour should be produced when the precipitate produced by ferric chloride is heated.

Hexyl-Resorcinol (B.P.C.). $C_{12}H_{18}O_2 = 194.1$. M.p., not below 66°. Ash, not more than 0.1%. The U.S.P. XII substance, dried to constant weight over sulphuric acid, contains 98% of $C_{12}H_{18}O_2$.

A tentative method for the determination of hexyl-resorcinol is described in *Methods of Analysis (A.O.A.C., 1940, 609)*. Dissolve about 0.8 g., accurately weighed, in 10 ml. of methyl alcohol in a 150 ml. glass-stoppered flask. Add 30 ml. of N/10 bromide-bromate solution, and 5 ml. of hydrochloric acid. Stopper the flask and swirl under running cold water to cool to room temperature, and continue to shake for 5 minutes. Add 5 ml. of 20% potassium iodide solution, swirl gently, wash the stopper with water, add 1 ml. of chloroform, and titrate with N/10 sodium thiosulphate. Towards the end of the titration add starch paste. The end-point is reached when the blue colour does not return during 30 seconds vigorous shaking. 1 ml. of N/10 bromide-bromate = 0.00488 g. of hexyl-resorcinol.

Pyrogallol (B.P.C. Supp. IV). $C_6H_6O_3 = 126.0$. M.p., 129° to 135°. Ash limit, 0.1%. The aqueous solution is clear, not more than slightly yellow, and neutral to methyl orange. The N.F. VII substance melts between 130° and 133°, and leaves not more than 0.1% of ash.

Resorcinol (B.P.). $C_6H_6O_2 = 110.0$. M.p., 110° to 111°. Ash limit, 0.05%. A 5% solution produces no precipitate with lead acetate solution, indicating absence of catechol. Resorcinol, U.S.P. XII, after drying to constant weight over sulphuric acid,

contains not less than 99.5% of $C_6H_4(OH)_2$. Assayed by bromine absorption during 1 minute, interaction with potassium iodide and titration with N/10 sodium thiosulphate.

Estimation of resorcinol in Resorcinol Ointment, *B.P.C.*, and Compound Resorcin Ointment, *B.P.C.*, by bromate titration.—Garratt, *Quart. J. Pharm.*, 1935, 472.

Sodii Phenolsulphonas (*B.P.C.*). $C_6H_5O_4SNa, 2H_2O = 232.1$. Assayed on its power of bromine absorption it contains from 99% to the equivalent of 103% of the pure substance.

Zinci Phenolsulfonates (*N.F. VII*). Contains 73.7 to 77.4% of anhydrous zinc phenolsulfonate, corresponding to not less than 99.5% of the octahydrate; determined by precipitation as carbonate and ignition to oxide.

An official method for the determination of phenolsulphonates is described in *Methods of Analysis* (*A.O.A.C.*, 1940, 623).

Dinitrophenol. 2:4-Dinitrophenol may be detected by the following tests. Add sodium hydroxide solution to the suspected sample and extract with chloroform. Allow the chloroform to evaporate and to the residue add 2 ml. of 10% sulphuric acid and 0.2 g. of powdered zinc; a pink colour developing within 10 minutes indicates the presence of dinitrophenol. Confirm by adding 10 drops of 1% sodium nitrite solution, allowing to stand in the dark for 5 minutes and adding 10 ml. of ether; a pink or violet colour should develop in the ethereal layer.—J. S. Shupe, *J. Ass. off. Agric. Chem., Wash.*, 1935, 464.

Official methods for the determination of dinitrophenol or its sodium compound in the absence and presence of interfering substances are described in *Methods of Analysis* (*A.O.A.C.*, 1940, 608).

Trinitrophenol (*B.P.*). $C_6H_3O_7N_3 = 229.0$. Contains not less than 99% of $C_6H_3O_7N_3$. M.p., 121° to 123° (precautions being taken). Residue on extraction with benzene at 50° , not more than 0.1%. Determined by titration with sodium hydroxide to phenolphthalein. The *U.S.P. XII* allows 0.2% of matter insoluble in benzene at ordinary temperature and dried at 100° . Acidum picricum, *P. Helv. V*, is assayed by dissolving 0.29 g. in 30 ml. of water and adding 10 ml. of a solution containing 0.36% of potassium iodate and 1.5% of potassium iodide. When the potassium picrate formed has dissolved, titrate with N/10 sodium thiosulphate, using starch as indicator towards the end of the titration and making a blank on the iodate-iodide solution. 1 ml. of N/10 $Na_2S_2O_3, 5H_2O$ is equivalent to 0.022905 g. of $C_6H_3O_7N_3$.

Estimation of Picrates. The picrate is dissolved in water and titrated with 0.001N methylene blue solution. The methylene blue picrate, formed during the titration, is extracted with chloroform from the aqueous layer. Since methylene blue is practically unextractable with chloroform, the end-point is detected by the colour of the aqueous layer, the titration liquid acting as indicator. The method is also used for the estimation of picrolonates.—A. Bolliger, *Analyst*, 1939, 416.

Carbasus Trinitrophenolis (*B.P.C.*). By titration of the gauze shaken with water a content of from 1.5 to 2.5% *w/w* of $C_6H_3O_7N_3$ should be indicated.

"T.N.T." *Trinitrotoluene*. *Syn.* Trotyl. $C_6H_5CH_3(NO_2)_3 = 227.1$. Commercially it is seen as crumbs or granules of fine plate crystals. Melting about 80° . It is obtained by nitrating toluene. Soluble in acetone, ether, benzene, and xylol.

"T.N.T." is stable and does not attack metals. When absorbed, mainly through the skin, it is liable to cause dermatitis, digestive troubles, cyanosis and jaundice.

Amatol is T.N.T. mixed with 40 to 60% of ammonium nitrate. Mixed with 20% it is AMMONAL.

For further information on T.N.T. and other explosives see "High Explosives,"—E. de W. S. Colver, 1918; "T.N.T. and Mono- and Di-Nitrotoluene, their manufacture and properties."—G. Carlton Smith, 1918; and "Explosives," by E. de Barry Barnett, 1919.

Seven cases of T.N.T. jaundice in an ordnance filling factory, with two deaths, the jaundice appearing within six months of beginning work. The associated symptoms were nausea, anorexia and epigastric discomfort. Treatment was by glucose, insulin and vitamin C. Prophylactic measures include protection of the skin, washing, adequate ventilation, a healthy life and full diet.—R. M. Evans, *Lancet*, ii/1941, 552.

The T.N.T. health hazard.—H. M. Roberts, *Brit. med. J.*, ii/1941, 647.

Clinical manifestations of tetryl and trinitrotoluene.—J. Hilton and C. N. Swanson, *Brit. med. J.*, ii/1941, 509.

Trinitro-butyl-toluene, $C_8HCH_3C_6H_2(NO_2)_3$, is synthetic musk or tonquinol.

In artificial musks, it is possible to replace the nitro groups by CHO, OCH₃, COCH₃, halogens and CN, without altering the odour, but the tertiary butyl group, or the presence of tertiary carbon, is essential.—*Per um. essent. Oil Rec.*, 1924, 360.

PHENOLPHTHALEINUM

Phenolphthaleinum (*B.P. Add. I*). $C_{20}H_{14}O_4 = 318.1$. M.p., not below 258°. Sulphated ash, not more than 0.05%. The *U.S.P. XII* requires the m.p. to be not below 258°; ash, not more than 0.1%.

Determination in Tablets. A method for determination of phenolphthalein in tablets is given in the *N.F. VII*. A portion of powdered tablets is extracted with boiling alcohol, cooled, adjusted to volume, filtered, and an aliquot part evaporated to dryness. Four or more drops of potassium hydroxide solution and 5 ml. of water are added and the phenolphthalein dissolved completely. Maintaining the temperature at 0° to 5°, 4.5 ml. of an iodine solution (7 g. of iodine in 10 ml. of saturated potassium iodide solution, water to 60 ml. and potassium hydroxide solution to discharge the colour) is added, followed by hydrochloric acid to complete precipitation. The precipitate is dissolved in potassium hydroxide solution, the precipitation and resolution repeated twice and then sodium sulphite solution (15%) added and if necessary filtered. After acidifying with hydrochloric acid and heating on a water-bath for 30 minutes, the precipitate is collected, washed with cold water and petroleum ether and dried at 130° to 140°. 1 g. of residue = 0.3871 g. of phenolphthalein.

Determination in tablets may be accomplished by extraction in a Soxhlet apparatus with acetone, from which the phenolphthalein is removed by shaking with a known volume of N/10 sodium hydroxide. The latter is filtered, an aliquot portion is acidified and the phenolphthalein extracted with ether. The ethereal solution is washed with water and evaporated, and the residue dried and weighed.—G. Thomas, *J. Pharm. Belg.*, 1939, 21, 361.

An official quantitative ether-extraction method for the determination of phenolphthalein in tablets and a tentative method for chocolate preparations similar to the above *N.F. VII* process are described in *Methods of Analysis* (*A.O.A.C.*, 1940, 611). A tentative method for the determination of acetylsalicylic acid and phenolphthalein tablets is also described (*ibid.*, 571).

The second report of the Commission Internationale des Spécialités includes an agreed method for the determination of phenolphthalein in tablets in which the phenolphthalein extracted with hot alcohol 95% is dissolved in a little 50% potassium hydroxide solution and converted into tetraiodophenolphthalein by adding N/10 iodine solution in the presence of ice, the excess of iodine being titrated with sodium thiosulphate solution.—*Bull. Féd. int. Pharm.*, 1938, 87.

Determination in Paraffin Emulsions. A sufficient quantity of emulsion to yield 0.05 to 0.1 g. of phenolphthalein is accurately weighed into a glass-stoppered Erlenmeyer flask, a mixture of 75 ml. of ether and 25 ml. of alcohol is added, and the mixture is shaken vigorously and then set aside for 30 minutes.

It is then filtered into a separating funnel and the precipitated agar is washed with successive portions of the same ether-alcohol solvent mixture. The agar is redissolved in about 5 ml. of water and again precipitated with the solvent mixture, the liquid and washings being filtered into the separating funnel containing the filtrate previously obtained. The agar may be tested for phenolphthalein by addition of alkali to an aqueous solution of a small portion and is discarded if no phenolphthalein is present. The phenolphthalein is completely extracted from the solvent mixture by shaking with successive 10 ml. portions of N/25 sodium hydroxide, and precipitated by slowly adding N/10 sulphuric acid in slight excess. It is then extracted from the acid liquid by means of ether, re-transferred to N/25 sodium hydroxide, and again precipitated by means of a very small excess of N/10 acid. The liquid is allowed to stand overnight to allow large crystals to form and the phenolphthalein is collected on a tared Gooch crucible, washed with 4 portions, each of 5 ml. of water, dried at 100° and weighed. The filtrate and washings are transferred to a 250 ml. graduated flask, the beaker in which the phenolphthalein was precipitated is rinsed out with the N/25 alkali which was added to that in the flask, the contents of which are made up to volume. The phenolphthalein remaining in solution is now determined colorimetrically using as standard a solution of 0.03 g. in 250 ml. of N/25 sodium hydroxide, the weight so obtained being added to that previously obtained.—C. F. Bickford and R. E. Schoetzw, *J. Amer. pharm. Ass.*, 1936, 1128.

Fluoresceinum Solubile (B.P.). $C_{20}H_{10}O_5Na_2 = 376.1$. Soluble fluorescein loses at 105° not more than 5%.

Iodophthaleinum (B.P.). $C_{20}H_8O_4I_4Na_2 \cdot 3H_2O = 919.8$. Contains not less than 85% of phthalein, and the separated phthalein contains 61 to 62% of I. Assayed by precipitation of the phthalein from aqueous solution with dilute hydrochloric acid, washing with a mixture of equal volumes of acid and water, and drying at 110°; by fusion of a portion of the residue with sodium carbonate, extraction with hydrochloric acid and water, and titration with M/20 potassium iodate the iodine content is obtained. Iodophthaleinum Sodicum, *U.S.P. XII*, contains not less than 85% of tetraiodophenolphthalein and this contains 60 to 63% of I. The tetraiodophthalein obtained in the assay is tested for proportion of iodine by dissolving on the steam-bath in sodium hydroxide solution (5%) and, after the addition of potassium permanganate solution (1 in 15), heating on the steam-bath for three-quarters of an hour. After cooling, the mixture is diluted with water, diluted sulphuric acid and sodium bisulphite solution (20%) added, followed by potassium permanganate solution till a faint yellow colour appears and the colour finally discharged with sodium bisulphite solution. After addition of glacial acetic acid and then ammonium carbonate the mixture is titrated in diffuse light with N/10 silver nitrate using diiodofluorescein as indicator. The *Fr. Cx.* 1937 determines iodophthalein similarly; it should contain not less than 85%; iodine content not less than 52% determined by the process of the *Fr. Cx.* 1937 described under Thymol Iodide.

The use of this compound as a diagnostic test for cholecystic disease is described on p. 1040.

Assay. The following assay is recommended by the Sub-Committee on Synthetic Chemicals of the Committee on Pharmaceutical Chemistry:—Mix about 0.3 g., accurately weighed, of the phthalein, obtained in the assay for phthalein, with 2 g. of anhydrous sodium carbonate; place in a small crucible, and then fill the crucible completely with anhydrous sodium carbonate well

pressed down; invert the crucible and contents in a larger crucible, and add sufficient anhydrous sodium carbonate to seal the junction of the two crucibles. Heat rapidly and strongly over a Bunsen flame, and continue the heating for twenty minutes; allow to cool, and dissolve the residue in 100 ml. of hot water; filter, and wash the filter with a little water. To the cooled solution add hydrochloric acid until effervescence ceases, then add an additional 10 ml. of hydrochloric acid and 10 ml. of solution of potassium cyanide and titrate with M/20 potassium iodate until the dark brown solution which is formed becomes light brown, then add 5 ml. of mucilage of starch and continue the titration until the blue colour disappears. Each ml. of M/20 potassium iodate is equivalent to 0.01269 g. of I.—(*British Pharmacopœia Commission Report*, No. 12, May 1939.)

Sulfobromophthaleinum Sodicum (U.S.P. XII). $C_{20}H_8Br_4O_{10}S_2Na_2 = 838.04$. Loses at 100° not more than 5%. A test for sensitivity is included; 0.2 ml. of 0.1% solution diluted to 50 ml. with freshly boiled and cooled distilled water produces a strong violet colour upon addition of 0.2 ml. of N/50 sodium hydroxide, and the colour is discharged upon adding 0.2 ml. of N/50 sulphuric acid.

Phenol-Rubrum (B.P.C.). $C_{19}H_{14}O_5S = 345.2$. Phenol red loses at 110° not more than 1%. Sulphated ash, not more than 0.2%. The U.S.P. XII limit of moisture in Phenolsulfophthaleinum is the same, and the ash not more than 0.2%; a test for sensitiveness and a limit of matter insoluble in 2.5% sodium bicarbonate solution, dried at 110° , of not more than 0.2% are included.

For the use of phenol red as a renal function test see p. 663.

PHYSOSTIGMINA

Physostigminæ Salicylas (B.P.). $C_{15}H_{21}O_2N_3, C_7H_6O_3 = 413.2$. M.p. of the dry substance, 185° to 187° . Loses, when dried at 100° , not more than 1%; ash, not more than 0.1%. 0.1 g. complies with a limit test for readily carbonisable substances. The U.S.P. XII substance contains not more than 2% of moisture and complies with a test for sulphate.

Physostigminæ Sulphas (B.P.C.). $(C_{15}H_{21}O_2N_3)_2, H_2SO_4 = 648.5$. M.p., after drying at 100° , about 145° . When dried at 100° , loses not more than 1%. Ash, not more than 0.1%. Complies with tests for readily carbonisable substances and salicylate.

Neostigminæ Bromidum (U.S.P. XII). $C_{12}H_{19}BrN_2O_2 = 303.2$. Loses not more than 2% at 100° and then contains not less than 98% of $C_{12}H_{19}BrN_2O_2$. M.p. with decomposition, about 167° ; ash, not more than 0.15%. Assayed by hydrolysis with sodium hydroxide solution and distillation of the volatile amine into excess N/10 sulphuric acid followed by back titration of the excess acid, using methyl red indicator.

Tabellæ Neostigminæ Bromidi (U.S.P. XII). Contain 93 to 107% of the labelled amount of $C_{12}H_{19}N_2O_2Br$, including all tolerances. Assayed by macerating a weighed quantity of powdered tablets with 70% alcohol containing 1% of dilute hydrochloric acid, decanting through a filter and extracting the residue with two further portions of alcohol, removing most of the alcohol, transferring the residual solution to a Kjeldahl flask, adding water and sodium hydroxide solution and distilling into excess N/10 sulphuric acid, finally titrating the excess acid with N/10 sodium hydroxide, using methyl red indicator.

Neostigminæ Methylsulphas (*U.S.P. XII*). $C_{15}H_{22}N_2O_6S = 334.4$. Assayed by the *U.S.P. XII* method for neostigmine bromide and complies with the same standard. Loss at 100° , not more than 1%; m.p., 142° to 145° . (Neostigmine is known in this country under the trade name of Prostigmin—see Vol. I, p. 821.)

PILOCARPINA

Pilocarpinæ Hydrochloridum (*B.P.C.*). $C_{11}H_{16}O_2N_2.HCl = 244.6$. M.p., 204° to 205° , after drying to constant weight at 100° . Specific rotation, determined on a 10% w/v aqueous solution, $+90^\circ$ to $+92^\circ$. Ash, not more than 0.1%. Complies with tests for foreign alkaloids.

An official method for the determination of pilocarpine hydrochloride in tablets is described in *Methods of Analysis* (A.O.A.C., 1940, 588). The pilocarpine, extracted with chloroform from ammoniacal solution, is dissolved in an excess of standard sulphuric acid and back-titrated with standard sodium hydroxide, using methyl red as indicator.

Pilocarpinæ Nitras (*B.P.*). $C_{11}H_{16}O_2N_2.HNO_3 = 271.2$. M.p., 174° to 178° . Specific rotation of the 10% w/v aqueous solution, $+77^\circ$ to $+83^\circ$. Ash, not more than 0.1%. Complies with tests for certain other alkaloids. The *U.S.P. XII* requires the ash from 0.2 g. to be negligible, and the salt must comply with a test for various foreign alkaloids; 0.1 g. complies with a test for carbonisable substances.

A colorimetric method for the determination of pilocarpine in hypodermic tablets and injections, depending upon Shupe's modification of Helch's identity test, is described. Tables are given relating the value of the blue component of the colour obtained to the alkaloidal content.—N. L. Allport and N. R. Jones, *Quart. J. Pharm.*, 1942, 247.

Jaborandi (*B.P.C.*). Consists of the dried leaflets of *Pilocarpus microphyllus* Stapf, and contains not more than 5% of stalks, stems and other foreign organic matter.

P. microphyllus is largely used in making pilocarpine and was official in *U.S.P. IX* if yielding not less than 0.6% of alkaloids.

PIPER NIGRUM

Piper Nigrum (*B.P.C.*). Contains not more than 2% of foreign organic matter. Ash, not more than 6%. Acid-insoluble ash, not more than 1%.

The Food and Drug Administration of the U.S. Dept. of Agriculture defined black pepper as the dried immature berry of *Piper nigrum* L., containing not less than 6.75% of non-volatile ether extract, not less than 30% of starch, not more than 7% of total ash and not more than 1.5% of ash insoluble in hydrochloric acid. **Ground black pepper**: the product made by grinding the entire berry of *Piper nigrum* L., containing the several parts of the berry in their normal proportions. **Long pepper**: the dried fruit of *Piper longum* L. **White pepper**: the dried mature berry of *Piper nigrum* L. less the outer, or outer and inner, coatings: containing not less than 7% of non-volatile ether extract, not less than 52% of starch, not more than 5% of crude fibre, not more than 3.5% of total ash, and not more than 0.3% of ash insoluble in hydrochloric acid.—S. R. A., F.D., No. 2, Rev. 5, Nov. 1936.

Cubeba (*B.P.C.*). Should contain not more than 7% of shrivelled immature fruits, rachis and stems, and not more than 2% of foreign organic matter. Ash limit, 8%. *Cubeba, N.F. VII*, should yield not less than 13% *v/w* of volatile oil; shrivelled fruits or stems, not more than 5%; foreign organic matter, not more than 2%; acid-insoluble ash, not more than 2%.

Genuine cubebes yield 6.44 to 8.47% of resin and 12.5 to 20% of essential oil. Adulteration can be detected by means of the physical characters of the oil. —J. F. Clevenger, *J. Ass. off. agric. Chem., Wash.*, 1937, 140.

Oleum Cubebæ (*B.P.C.*). Sp. gr., 0.910 to 0.930; α_D , -20° to -35° ; n_{D20° , 1.480 to 1.502. Not less than 60% distils between 250° and 280° .

PITUITARIUM

Pituitary (posterior lobe) Extract. This extract is controlled by the Therapeutic Substances Act 1925, and is official in the *B.P.* and as *Injectio Pituitarii Posterioris* in the *U.S.P. XII*.

Its potency can be determined by its stimulant action on the isolated uterus of the guinea-pig, which is known as its oxytocic property, by its pressor action on the blood pressure of the spinal cat, and also by its antidiuretic effect on rats. An international standard has been adopted consisting of a quantity of dry acetone-extracted posterior lobe material which is kept in the National Institute for Medical Research, Hampstead. The unit is the amount of activity present in 0.5 mg. of the standard powder.

Oxytocic Potency. The determination of the action on the uterus is made by excising one horn of the uterus of a virgin guinea-pig and suspending it in a bath of Ringer's solution which is oxygenated and maintained at 37° . The muscle is very thin and, when pituitary extract is added to the bath, quickly responds by contraction. The extent of the contraction is proportional to the dose, hence the potency of an unknown solution can be found by comparing the contraction which a given amount of it produces with the contraction produced by a given amount of the standard extract. In practice, difficulties arise because the muscle of the horn changes in sensitiveness and wrong conclusions may easily be drawn respecting the potency of an unknown solution. The test usually takes one or two days to complete.

Pressor Activity. The determination is made on the blood pressure of a spinal cat, prepared as described under Adrenaline (*q.v.*, p. 55). An intravenous injection of pituitary extract causes a rise of blood pressure not so rapid as that produced by adrenaline but lasting much longer. The height of the rise is proportional to the dose and an unknown preparation is examined to see what amount produces the same rise as that produced by a given amount of the standard extract. The test takes much time, as successive doses must be separated by an interval of 1 hour; this is necessary because successive injections of the same dose at shorter intervals produce a diminishing response.

Antidiuretic Activity. A reliable method of determining activity which is economical in skilled labour depends on the antidiuretic effect which may be observed in rats. If a group of four rats, kept without food overnight, is given water by stomach tube, in a dose of 5 ml. per 100 g. body weight, then a diuresis occurs which reaches a maximum in 60 to 90 minutes. If a dose of pituitary extract be given at the time the water is administered, the excretion of water is delayed and the maximum diuresis may not occur until 130 or 190 minutes according to the dose. To compare an unknown extract with the standard, sixteen rats receive water and eight of these are injected with standard extract and eight with the unknown extract. The time of maximum diuresis is determined for each group of eight rats. Two days later the experiment is repeated, reversing the groups, so that the eight rats which previously received the standard now receive the unknown and *vice versa*. The antidiuretic effect of the standard and of the unknown extract is now known for all of the sixteen rats. From these measurements the potency of the unknown extract can be expressed in terms of that of the standard by using a predetermined curve relating antidiuretic effect to dose. The antidiuretic potency of different commercial extracts expressed in units is almost always the same as the oxytocic potency expressed in units.

Gonadotrophic Hormone. The following assay methods are available. (1) *Increase in weight of seminal vesicles:* This is applicable to preparations of the gonadotropin in the urine of pregnant women and of that in pregnant mare's serum. It is completely objective; the sensitivity is moderate; and the process is simple. (2) *Increase in weight of ovaries:* Applicable to preparations of gonadotropins from all three sources and is completely objective. The sensitivity varies with the gonadotropin concerned, being low for that of pregnant mare's serum but higher with larger doses, and moderate for anterior pituitary gonadotropin. (3) *Increase in weight of uteri:* Applicable to all three types, is completely objective and about as easy as the previous ones. The sensitivity is great, a six-fold increase in weight being obtained on either pregnancy urine or pregnant mare's serum preparations, with doses which will only double the weight of the seminal vessels and hardly affect the ovarian weight at all. (4) *Vaginal cornification:* This method is the simplest and is applicable to all three types. It is reasonably objective provided one sets as his standard a full oestrus smear, i.e., the complete disappearance of leucocytes and their replacement by epithelial cells. The sensitivity is great and appears to parallel rather closely the uterine weight method. (5) *Luteinisation:* This method is unsatisfactory in practically every respect. Unless microscopic sections are prepared which takes time and labour, the degree of luteinisation is difficult to determine; macroscopic examination fails on the score of objectivity. The method is insensitive and individual variation is still rather great.

Of these methods vaginal cornification is simplest and reasonably objective with practice. Since both uterine weight and vaginal oestrus can be determined simultaneously, i.e., on the same animals, a combination of these two methods might be employed. It must be remembered, however, that both responses are also produced by oestrogens, and the absence of the latter from the material being assayed must be assured.—R. G. Gustavson and F. E. D'Amour (Council on Pharmacy and Chemistry, A.M.A.), *J. Amer. med. Ass.*, ii/1941, 188.

The interpretation of assays for gonadotropin in menstruating women is not, as yet, clear of confusing elements, though they are of some value in regard to non-pregnant women with amenorrhœa. It is well-established that in the complete absence of ovarian function in adult women there is an increased excretion of gonadotropin. Thus, in amenorrhœa due to ovarian failure, spontaneous menopause or surgical castration increased amounts of gonadotropin

are present in blood and urine. The increase in urinary gonadotropin may be detected within as little as three days following ovariectomy. Even in patients without ovaries, however, the excretion fluctuates significantly, necessitating repeated tests at times before conclusions can be drawn as to whether or not any particular patient has ovarian failure. Such tests are of some value, therefore, in determining whether the pituitary or the ovary is at fault in cases of amenorrhœa. If the absence of menstruation is due to failure of the pituitary little or no gonadotropin will be excreted; if the ovaries are at fault this substance will be found in most cases on thorough investigation.—S. C. Freed, *J. Amer. med. Ass.*, ii/1941, 104.

The luteinising hormone can be estimated by the method of Janssen and Loeser, *Arch. exp. Path. Pharmac.*, 1930, 151, 188, in which infantile rats are injected and the ovaries are examined for corpora lutea. Hill, Parkes and White (*J. Physiol.*, 1934, 81, 335) have proposed a method of estimating the ovulation-producing substance by means of this property of causing ovulation in the oestrous rabbit. The method depends on the fact that ovulation never occurs spontaneously in the rabbit but only after copulation or after injection with the hormone. In the adult female non-pregnant rabbit carefully segregated from the male, the ovaries always contain eggs waiting to be discharged. Full-grown female rabbits (3 to 4 kg.) may be used, which, when received from the dealer, must be kept for 5 weeks in order that pregnant animals may complete pregnancy and regain the oestrous condition. The extract to be tested is then injected into a batch of twenty rabbits, the dose being administered intravenously. On the following day the ovaries are examined by making incisions into the abdominal cavity under ether anaesthesia, using full aseptic precautions. The number of animals which have ovulated is thus determined. When this number is expressed as a percentage of the number of animals injected, the amount of extract containing 1 unit, which is the amount producing a 50% response, may be calculated from a curve relating dose to percentage of animals responding, which has been determined.

Thyrotrophic Hormone. The amount excreted is apparently increased following thyroidectomy. The use of thyrotrophic hormone assays in the clinic has been somewhat limited owing to the variability of the results of different investigators. In myxœdema an increased elimination has been observed. The blood of such patients is also claimed to be rich in this substance, while little, if any, is found in the opposite state of hyperthyroidism. With regard to acromegaly there is a difference of opinion as to whether there is increased excretion, while in Simmonds' disease it is claimed to be below normal. Practical use of this test is not very great, since considerable technical facilities are required. A further complication has arisen, since recent work indicates that the thyrotrophic principle may be separated into two factors, one which stimulates the growth of the thyroid gland and one which stimulates the secretion of the thyroid hormone.—S. C. Freed (Council on Pharmacy and Chemistry, A.M.A.), *J. Amer. med. Ass.*, ii/1941, 104.

PIX LIQUIDA

Pix Liquida (B.P.). Wood tar, known in commerce as Stockholm tar. *Pix Pini, U.S.P. XII*, leaves not more than 0.3% of ash. *Goudron Végétal, Fr. Cx.* 1937, by distillation with xylene, should yield not more than 2.5% of water, and loses not more than 18 to 19% at 100°. Acidity (expressed as acetic acid), not more than 0.1%. Saponification value, 115 to 120. Viscosity, 37.7 at 40° (Engler), 280° to 305° (Stokes).

Oleum Picis Rectificatum (U.S.P. XII). Sp. gr. at 25°, 0.960 to 0.990.

Pix Carbonis (B.P.C.). Ash, not more than 2%. Water shaken with it gives an alkaline reaction.

Pix Carbonis Præparata (B.P.). Coal tar heated at 50° for 1 hour.

Oleum Cadinum (B.P.). Sp. gr., 0.975 to 1.010; n_{D20}° , 1.510 to 1.530. The presence of pine tar oil is excluded by the absence of green colour in the resin test when a petroleum extract is shaken with copper acetate solution. Pix Juniperi, *U.S.P. XII*, complies with a similar test for rosin or rosin oils, but with addition of ether to the petroleum ether layer after separation, and has a sp. gr. at 25° of 0.950 to 1.055. The *Fr. Cx.* 1937 requires Goudron de Cade to comply with limits for distillation—not more than 2% below 150°, not less than 50% between 150° and 200°, not less than 30% between 200° and 250° and 18% above 250°. A minimum limit of substances insoluble in caustic soda solution of 60% is required, and the cadinine hydrochloride separated from this should melt at about 118°; a 10% solution of the essence in acetic ether should have rotation not less than -4° in a decimetre tube.

Oleum Rusci (B.P.C.). Sp. gr., 0.920 to 0.955. Unsaponifiable matter, not less than 70%. A test for absence of fir tar is described. Oleum Betulae Empyreumaticum Rectificatum, *N.F. VII*, has a sp. gr. at 25° of 0.886 to 0.950 and complies with a test for distinction from oil of cade.

PLUMBUM

Plumbi Acetas (B.P. Add. I). $C_4H_5O_4Pb \cdot 3H_2O = 379.3$. Contains from 99.5% to the equivalent of 104.5% of the pure substance. Assayed by precipitation as oxalate and titration with N/10 potassium permanganate. Plumbi Acetas, *U.S.P. XII*, contains 85.31 to 89.57% of the anhydrous salt, corresponding to not less than 99.5% of the trihydrate; in assaying, the lead is precipitated by addition of excess N/10 potassium dichromate, and, after filtering, the excess dichromate is determined in the filtrate iodometrically.

Liquor Plumbi Subacetatis Fortis (B.P.). Assayed for total lead by precipitation with oxalic acid, decomposition of the washed precipitate with sulphuric acid and titration with N/10 potassium permanganate, it contains 19 to 21.5% *w/w*. Alkalinity corresponding to 10.2 to 11.6% *w/w* of PbO , determined by addition of N/1 sulphuric acid, adjusting to volume, and titration of an aliquot of the clear liquid with N/1 sodium hydroxide, using phenolphthalein indicator.

Plumbi Carbonas (B.P.C.). Assayed for lead content by precipitation of the acetic acid solution with ammonium oxalate and titration of the precipitate, decomposed by sulphuric acid, with N/10 potassium permanganate, not less than 79% of Pb should be indicated. Matter insoluble in diluted nitric acid, not more than 1%.

Plumbi Iodidum (B.P.C. Supp. IV). $PbI_2 = 461.1$. Assayed for halogen content by addition of excess silver nitrate and nitric acid to a solution in sodium hydroxide solution and back titration

with N/10 ammonium thiocyanate; after allowance for the chloride in the sodium hydroxide solution, a purity of not less than 95% is required.

Plumbi Monoxidum (B.P.). $PbO = 223.2$. Loses not more than 4% on ignition and then contains not less than 99% of PbO . Assayed by precipitation as oxalate and titration with N/10 potassium permanganate. **Plumbi Monoxidum, N.F. VII,** is assayed by precipitation with excess N/10 oxalic acid, filtration and titration of the excess acid in an aliquot part with N/10 potassium permanganate; a purity of the freshly ignited substance of not less than 97% is required. Loss on ignition, not more than 4%.

The B.P. methods for the assay of lead preparations are inaccurate. Alternative methods of assay, which combine sufficient accuracy with easy and rapid manipulation, are suggested, and also assay processes for the suppository and plaster.—S. Wetherell, *Quart. J. Pharm.*, 1935, 453.

Determination of Lead in Organic Material. Lead in organic materials may be determined by using diphenylthiocarbazon (syn. dithizone), $C_6H_5 \cdot NH \cdot NH \cdot CS \cdot N : N \cdot C_6H_5$. The reagent is used as a 0.1% solution in chloroform; the solution should be freshly prepared. The organic material is oxidised by heating with sulphuric and nitric acids or with sulphuric acid and hydrogen peroxide and the excess oxidising agent is removed by evaporation. The cooled residue is diluted and 2 g. of citric acid is dissolved in the solution to prevent precipitation of iron. About 1 ml. of 10% potassium cyanide solution is added and the mixture made alkaline with ammonia. The lead is then extracted by shaking with three successive portions of the reagent (10, 5 and 5 ml.), each extract being washed once with 10 ml. of water. The mixed extracts are evaporated to dryness and the organic matter is destroyed by heating with 1 ml. of sulphuric acid until white fumes are evolved, then adding a few drops of nitric acid and continuing to heat until the liquid is almost colourless. The residue is dissolved in water and the lead determined colorimetrically in the usual manner by comparing the colour produced on adding sodium sulphide in the presence of ammonium acetate with that obtained with known amounts of lead.

Separation of lead from other metals, except bismuth, by extraction with diphenylthiocarbazon in chloroform, in the presence of potassium cyanide.—Allport and Skrimshire, *Analyst*, 1932, 440; also Roche Lynch, Slater and Osler, *Analyst*, 1934, 787. In presence of bismuth.—Hamence, *Analyst*, 1933, 46; 1934, 274.

LEAD POISONING

Lead Paint Regulations 1927 (S.R. & O., 1927, No. 847), under Lead Paint (Protection against Poisoning) Act 1926 (16 and 17 Geo. 5, c. 37).

Lead paint for buildings must be in paste form or paint ready for use (but red lead may be had raw or dry for stopping or filling). Must not be used in form of spray in interiors. Surfaces other than iron or steel must not be rubbed down or scraped by dry process, except if the surface contains no lead.

Spraying Paint or Lacquer. The practice of spraying paints or lacquer containing over 1% of lead should be discontinued, or carried out only where adequate air movement is provided. Similarly, the amount of benzol used in lacquers should be limited to 0.5%. Definite silicosis hazard from spraying of vitreous enamels.—*Lancet*, ii/1928, 177.

An improved method for determining the lead content of blood and excreta.—S. L. Tompsett and A. B. Anderson, *Lancet*, i/1939, 599.

A photometric method based upon the dithizone "mixed colour" method of Clifford and Wichman is proposed, which includes a three-stage extraction process, whereby the lead is obtained in a high state of purity.—D. M. Hubbard, *Industr. Engng Chem. (anal. Edn.)*, 1937, 29, 493.

The error in the above process decreases as the initial volume of material used decreases. This was found by comparison with the spectrographic method which also gives more accurate results with amounts of lead less than 1 microgramme. Both methods give good results for lead in fæces and mixed food

samples.—J. Cholak, D. M. Hubbard, R. R. McNary, and R. V. Storey, *Industr. Engng Chem. (anal. Edn.)*, 1937, 29, 488.

The determination of traces of lead in bone and biological materials can be made without ashing the organic material by the use of diphenylthiocarbazon to extract the lead. Fresh bone contains from 14.5 to 146 parts per million of lead; teeth from five normal persons contained 42.5 to 247.5 parts per million.—G. Roche Lynch, R. H. Slater and T. G. Osler, *Analyst*, 1934, 787.

Lead Tetraethyl is a liquid boiling at 150° with decomposition. It is manufactured by treating lead tetrachloride with methyl magnesium iodide. The products of combustion are: (1) with excess air—chiefly carbon dioxide and lead oxide, (2) with deficiency of air (e.g., in engine cylinder)—chiefly carbon monoxide and lead. It is not very volatile at ordinary temperatures.—*Pharm. J.*, i/1928, 268.

Lead poisoning due to the manufacture, etc., of tetraethyl lead. The condition bears little resemblance to clinical types of lead poisoning. The compound is not immediately corrosive, but causes necrosis after lengthy exposure. If allowed to remain on the skin for half an hour no sensation is produced but desquamation occurs after a day or two. Poisoning results from a combination of skin absorption and inhalation of vapour. The inhalation of dust has also caused illness.—R. A. Kehoe, *J. Amer. med. Ass.*, ii/1925, 108.

Determination in motor fuels of lead tetraethyl.—*Analyst*, 1926, 104.

Interim Report of Departmental Committee of Min. Health (Report issued July 27th, 1928). There is no evidence that the use of ethyl petrol involves more danger to health than the ordinary, but the precautions suggested by the U.S. Committee should be carried out, e.g., labelling of cans and pumps, leaflets, and dyeing of the substance red. Warning against use for purposes other than as motor fuel, e.g., cooking and cleaning. In no case does the amount of lead tetraethyl in commercial spirit exceed 1 in 1300 by volume or 1 in 650 by weight. The deaths in the U.S.A. not attributed to the diluted mixture (ethyl petrol). Drivers and garage employees in U.S.A. gave no definite signs of poisoning after exposure for 2 years.—*Brit. med. J.*, ii/1928, 219.

PODOPHYLLUM

Podophyllum (B.P.). Contains not more than 2% of other organic matter. Podophyllum, *N.F. VII*, contains not more than 2% of foreign organic matter, not more than 2% of acid-insoluble ash and yields not less than 5% of resin of podophyllum; it is assayed by continuous extraction, followed by percolation and adjustment to volume with alcohol; an aliquot part is shaken with equal volumes of chloroform and hydrochloric acid (0.6%) and the acid layer extracted with three further portions of 1½ volumes of alcohol-chloroform (1:2); after shaking the alcohol-chloroform liquids with a further volume of the hydrochloric acid and washing this with three 1½ volume portions of alcohol-chloroform, the combined extractions are evaporated to dryness, 1 ml. of dehydrated alcohol added and dried at 80°.

A tentative method for the determination of resin in podophyllum is described in *Methods of Analysis (A.O.A.C., 1940, 598)*.

Podophyllum Indicum (B.P.). Contains not more than 2% of foreign organic matter.

T. A. Henry found that the action of both podophyllum and Indian podophyllum is due to podophyllotoxin (purgative) and podophylloresin (purgative and cholagogue). The Indian is richer in the former.

The following histological features of Indian podophyllum serve to distinguish it from the American drug: (1) The complete absence of epidermal cells with brown contents. (2) The presence of cluster crystals of calcium oxalate, which are comparatively few in number and do not exceed 60 microns in

diameter. (3) The almost complete absence of pericyclic fibres. (4) The presence of a fairly large amount of thin-walled, isodiametric cork cells. (5) The sclerenchymatous cells, which are irregular or somewhat contorted in form, are more abundant than in American podophyllum. As in the American drug, there is much starch, individual granules not exceeding 35 microns in diameter.—T. E. Wallis and S. Goldberg, *Quart. J. Pharm.*, 1937, 311.

Podophylli Resina (B.P.). Loss at 100°, not more than 5%. Ash, not more than 1%. Matter insoluble in dilute solution of ammonia, not more than 10% for resin from podophyllum, and not more than 50% for resin from Indian podophyllum. Resina Podophylli, *N.F. VII*, is obtained from *Podophyllum peltatum* only; ash, not more than 1.5%. A test for distinction from resin obtained from *Podophyllum Emodi* is included:—0.4 g. is added to 3 ml. alcohol (60%) and then shaken gently with 0.5 ml. of potassium hydroxide solution; the mixture should not gelatinise. Résine de Podophylle, *Fr. Cx.* 1937, yields not less than 75% of ether-soluble resins and not less than 65% of chloroform-soluble resins. Podophyllum, *P. Helv. V*, is the mixture obtained by precipitation with water from an alcoholic extract of the rhizome of *Podophyllum peltatum* containing not less than 40% of podophyllotoxin. *Assay.* Shake frequently during 30 minutes 0.45 g. in fine powder with 15 ml. of chloroform; transfer 10 ml. of the filtered solution (=0.3 g. of the drug) to a tared flask with 50 g. of light petroleum, collect the precipitate on a tared filter and wash the flask and filter with 20 ml. of light petroleum. Dry the flask and filter with the precipitate for 1 hour at 70° and weigh. The dried residue should weigh not less than 0.12 g.

POTASSII HYDROXYQUINOLINI SULPHAS

Potassii Hydroxyquinolini Sulphas (B.P.C.) may be a mixture of 8-hydroxyquinoline sulphate and potassium sulphate or of 8-hydroxyquinoline and potassium acid sulphate. It partly liquefies at 172° to 178° and yields from 30 to 33% of sulphated ash.

Oxychinolinum sulfuricum, *P. Helv. V*, is oxyquinoline sulphate containing not less than 95% of $(C_9H_7ON)_2H_2SO_4$. It is assayed by dissolving 0.5 g. in 250 ml. of water, mixing 50 ml. of the solution in a closed flask with 25 ml. of dilute hydrochloric acid and 22 ml. of N/10 bromate-bromide solution, and adding 1 g. of potassium iodide after 1 minute. The liberated iodine is then titrated with N/10 sodium thiosulphate in the usual manner and starch is used as indicator. Each ml. of bromate-bromide solution is equivalent to 0.0048525 g. of $(C_9H_7ON)_2H_2SO_4$.

Chiniofonum (B.P. Add. I) is a mixture of approximately 4 parts by weight of 7-iodo-8-hydroxyquinoline-5-sulphonic acid and 1 part of sodium bicarbonate. Contains 28.2 to 29.6% of I and 18 to 22% of $NaHCO_3$. Assayed for iodine by ignition with sodium carbonate, solution in water, filtration and neutralisation with sulphuric acid to methyl orange, followed by oxidation with

bromine and sulphuric acid, removing oxidising substances remaining in solution after boiling for 10 minutes with phenol in glacial acetic acid, finally titrating the iodine liberated on the addition of potassium iodide with N/10 sodium thiosulphate; 1 ml. of N/10 sodium thiosulphate = 0.002116 g. of I. Sodium bicarbonate determined by the interaction in a closed vessel for at least 12 hours of N/10 barium hydroxide and the carbon dioxide produced by interaction of the substance and water and afterwards with dilute hydrochloric acid and water, followed by back titration of the N/10 barium hydroxide with N/10 oxalic acid to phenolphthalein. Chiniofonum, *U.S.P. XII*, contains 26.5 to 29% of I and complies with tests for free iodine and iodide.

Assay of Chiniofonum, U.S.P. XII. Heat on a steam-bath 0.4 g. with 15 ml. of aqueous sodium hydroxide solution (1 in 20), contained in a 500-ml. Erlenmeyer flask; when completely dissolved, add 25 ml. aqueous potassium permanganate solution (1 in 15) and several small pieces of porcelain. After rinsing the inner wall of the flask with a small quantity of distilled water, place a small short-stemmed funnel in the mouth of the flask and heat on a steam-bath for 1 hour, rotating the flask at 5-minute intervals. Allow to cool to room temperature, and add 75 ml. of distilled water, followed by 20 ml. of dilute sulphuric acid. Rotate the flask and add, all at once, 15 ml. of aqueous sodium bisulphite solution (1 in 5). When the solution has become colourless, add potassium permanganate solution, drop by drop, until the liquid is faintly yellow. Immediately add sodium bisulphite solution, drop by drop, to discharge the colour. Add 2 ml. of glacial acetic acid, 1 g. of dextrin, 1 g. of freshly powdered ammonium carbonate, and 0.5 ml. diiodofluorescein (0.5% in 75% alcohol). Titrate against N/10 silver nitrate in diffused light until the colour changes from pale yellow to pink; 1 ml. of N/10 silver nitrate = 0.01269 g. of I.

Tabellæ Chiniofoni (U.S.P. XII). Contain iodine equivalent to 26 to 29% of the labelled amount of chiniofon, including all tolerances. A test for inorganic iodine is included. A weighed quantity of powdered tablets is assayed by the process described for chiniofon.

POTASSIUM

Notes on other potassium salts are included under the corresponding acids (see Index).

Potassii Bicarbonas (B.P.). $\text{KHCO}_3 = 100.1$. Contains from 99% to the equivalent of 100.5% of KHCO_3 . The *B.P. Add. I* modifies the limit test for iron by dissolving the potassium bicarbonate in 20 ml. of dilute nitric acid FeT . The *U.S.P. XII* substance should contain not less than 99% of KHCO_3 , after drying over sulphuric acid.

Potassii Carbonas (B.P. Add. I). Loses from 14 to 18% when dried at 200° to 300° , and then contains not less than 99% of K_2CO_3 . Potassii Carbonas, *U.S.P. XII*, dried to constant weight at 180° , contains not less than 99% of K_2CO_3 . Loss at 180° , 10 to 16.5%. Carbonate Neutre de Potassium, *Fr. Cx.* 1937, loses not more than 5% at red heat and contains 95% of the anhydrous salt.

Potassii Chloras (B.P.). $\text{KClO}_3 = 122.6$. Contains not less than 99% of the pure salt. Assayed by digestion, for 20 minutes at 50° , of a solution with acid ferrous sulphate solution and potassium iodide, followed by dilution and titration of the liberated

iodine with N/10 sodium thiosulphate, a blank titration being made. The *N.F. VII* assays by reducing a solution with acid ferrous sulphate solution by boiling for 10 minutes, manganous sulphate solution being added and the excess ferrous sulphate titrated with N/10 potassium permanganate, a blank determination being performed; a purity of 99% is required.

The following assay is recommended by the Sub-Committee on Inorganic Chemicals of the Committee on General Chemistry:—Dissolve about 0.3 g., accurately weighed, in 10 ml. of water in a stoppered flask, add 0.5 g. of sodium nitrite dissolved in 10 ml. of water, followed by 20 ml. of nitric acid; stopper the flask and set aside for ten minutes; add 100 ml. of water and sufficient solution of potassium permanganate to produce a permanent pink colour; decolorise by the addition of a trace of ferrous sulphate; add 30 ml. of N/10 silver nitrate, filter, and titrate with N/10 ammonium thiocyanate, using solution of ferric ammonium sulphate as indicator. Each ml. of N/10 silver nitrate is equivalent to 0.01226 g. of KClO_3 .—(*British Pharmacopœia Commission Report*, No. 14, September 1939.)

Tabellæ Potassii Chloratis (*N.F. VII*). Contain 94 to 106% of the labelled amount of potassium chlorate, including all tolerances. Assayed by dissolving in water, treating with acid solution of ferrous sulphate and titrating the excess ferrous sulphate with N/10 potassium permanganate.

Schulze's Maceration Mixture. A mixture of potassium chlorate 10 (moistened with water), with nitric acid 40; or a solution of 0.06 g. potassium chlorate in water 100 ml. and 1 ml. of nitric acid. For separation of muscle fibre in animal, and ligneous tissue in vegetable, histology. Distinguish from the following:—

Schulze's Chlor-Zinc-Iodine Reagent for Cellulose. Dissolve 110 g. of zinc in 300 ml. of pure hydrochloric acid, and evaporate to 150 ml. (sp. gr., about 1.8). Dissolve separately 12 g. of potassium iodide in as little water as possible; add 0.15 g. of iodine. Mix the solutions, and filter if necessary through asbestos.—Bower and Wynne Vaughan.

Tabellæ Potassii Permanganatis (*N.F. VII*). Tablets of 0.3 g. or more contain 94 to 106%, and tablets of less than 0.3 g. contain 92.5 to 107.5%, of the labelled amount of potassium permanganate, including all tolerances. Assayed by heating at 80° with excess N/10 oxalic acid in acid solution and back titrating with N/10 potassium permanganate.

Potassii Hydroxidum (*B.P. Add. I*). $\text{KOH} = 56.10$. Contains total alkali, by titration with N/1 sulphuric acid to methyl orange, equivalent to not less than 85% of KOH , and not more than 4% of K_2CO_3 . Determined by addition of barium chloride solution and titration with standard hydrochloric acid using phenolphthalein indicator, followed by further titration of the same liquid to the end-point of bromphenol blue; the second titration is equivalent to the K_2CO_3 and the sum of the titrations is equivalent to the total alkali. Potassii Hydroxidum, *U.S.P. XII*, contains not less than 85% of total alkali as KOH , of which not more than 3.5% is K_2CO_3 . Assayed by titration with N/1 sulphuric acid to phenolphthalein, continuing the titration to methyl orange. The *Fr. Cx.* 1937 titrates the total alkali to phenolphthalein and the hydroxide after precipitation of the carbonate with barium chloride; contains not less than 85% of hydroxide and not more than 2% of carbonate.

Liquor Potassii Hydroxidi (*B.P.*). Contains from 4.75 to 5.25% w/v of total alkali, calculated as KOH . Liquor Potassii Hydroxidi, *N.F. VII*, contains from 4.5 to 5.5% w/v of total alkali, calculated as KOH , of which not more than 0.35% w/v is K_2CO_3 .

Detection and Determination of Potassium. Potassium may be detected and determined by means of sodium 6-chloro-5-nitrotoluene-3-sulphonate, $\text{CH}_3\cdot\text{C}_6\text{H}_4\cdot\text{NO}_2\cdot\text{Cl}\cdot\text{SO}_3\text{Na}$. The reagent is used as a 10% aqueous solution and yields a white crystalline precipitate, either at once or on standing, with solutions of potassium compounds. To a solution containing about 0.1 to 0.2 g. of potassium in 10 ml. 50 to 100% excess of the reagent is added. The whole is heated to the boiling-point and the conditions should be such that the potassium salt is just dissolved, water being added or the solution evaporated as may be necessary. The liquid is allowed to stand overnight at a temperature not exceeding 5° , and then filtered through a tared, sintered glass crucible, washed with a saturated aqueous solution of the potassium derivative of the reagent and, finally, rinsed with a small quantity of water, dried at 120° and weighed. Each gramme of precipitate is equivalent to 0.135 g. of K. The test solution must be free from barium, and ammonia, if present, must be removed by boiling with sodium hydroxide.

Determination of Potassium as Potassium Silver Cobaltinitrite. 2 ml. of a potassium solution free from halogens and containing from 0.03 to 2 mg. of potassium is acidified in a centrifuge tube with 1 drop of glacial acetic acid. 1 ml. of 0.7% silver nitrate is added and the solutions well mixed with a glass rod; 1 ml. of 80% acetone is added gradually and the tube and glass rod are then placed in an ice-bath for 5 minutes. 1 ml. of freshly prepared 25% sodium cobaltinitrite solution, also cooled in the ice-bath for 5 minutes, is added drop by drop with stirring after each addition; after 0.9 ml. of reagent has been added, the glass rod is allowed to drain and is then rinsed with the remaining 0.1 ml. The tube is kept in the ice-bath for 2 hours, then centrifuged at 3000 r.p.m. for 3 minutes. The clear liquid is decanted, the tube inverted to drain, and its mouth and upper part wiped with filter paper. 3 ml. of 50% acetone is added gradually down the sides of the tube in small quantities at a time, the tube again centrifuged and drained as before and then 3 ml. of 80% acetone is run in and the precipitate stirred with a glass rod which is drained and rinsed with 2 ml. of 80% acetone. The tube is centrifuged and the washing with 80% acetone and centrifuging are repeated twice. The potassium is determined indirectly by means of the nitrite content of the potassium silver cobaltinitrite complex. An excess of N/50 ceric sulphate is added to the dry precipitate followed by 2 ml. of 2N sulphuric acid, the tube is placed in a boiling water bath to complete the decomposition, and the contents transferred to an Erlenmeyer flask using further quantities of 2N sulphuric acid for rinsing. The flask is then boiled and cooled, the solution is treated with excess of N/50 ferrous ammonium sulphate and is then titrated with N/50 ceric sulphate using 0.1% aqueous solution of erioglaucine as indicator. 1 ml. of N/50 ceric sulphate corresponds to 0.1087 mg. of potassium. The accuracy of the method is within 1% and is not affected by the presence of other metals.—A. M. Ismail and H. F. Harwood, *Analyst*, 1937, 443.

Modifications are described of the cobaltinitrite method of estimating potassium, which render it applicable to urine, serum, etc., containing small amounts of potassium.—G. B. West, *Quart. J. Pharm.*, 1941, 26.

PYRETHRUM

Pyrethri Flos (B.P.C.). Contains not less than 0.4% of pyrethrin I. Ash limit, 8%. Acid-insoluble ash limit, 1%. Assayed by the B.P.C. '34 process: continuously extract 10 g. (in 85 powder) with petroleum (b.p. 40° to 50°); reflux the solution (measuring about 50 ml.) with 5 ml. of N/1 sodium hydroxide in methyl alcohol, in a micro-Kjeldahl apparatus, on a water-bath for 2 hours; acidify the cooled mixture with N/1 sulphuric acid and steam distil till 150 ml. of aqueous distillate is collected below the light petroleum; extract the whole distillate in a separator with the addition of 10 g. of sodium chloride, with the light petroleum and two further portions; the petroleum liquids and alcohol and water rinsings of the separator are titrated with N/50 sodium hydroxide

till a distinct pink colour is produced to phenolphthalein after vigorous shaking; deduct the blank titration required for the light petroleum; 1 ml. of N/50 sodium hydroxide is equivalent to 0.0066 g. of pyrethrin I.

The *Fr. Cx.* 1937 describes an assay process for pyrethrins I and II in *Chrysanthème Insecticide*; dried flowers of good quality should contain 0.5 to 1.2%; the powder is subjected to continuous extraction with light petroleum ether during 6 hours, the solvent evaporated and the residue dissolved in ether; after shaking with normal potash the ether is evaporated, the residue refluxed with N/1 potash in methyl alcohol, the alcohol evaporated and the residue dissolved in water. The solution is shaken with ether and alcohol and the ethereal layers treated with sodium chloride solutions. The filtered aqueous liquids rendered just acid with dilute hydrochloric acid are exhausted with ether, finally titrating the evaporated ethereal solutions with N/5 methyl-alcoholic potash to phenolphthalein. A physiological test is also included. *Flos Pyrethri, P. Helv. V*, yields to ether by continuous extraction not less than 5% of extractive; ash, not more than 8.5%.

Kenya pyrethrum is sold on a guaranteed pyrethrin content of 1.3%, as opposed to 0.9% in Japanese pyrethrum. Moisture content of Kenya flowers runs from 5 to 7%; Japanese flowers to close on 15%. In Kenya the production is scientifically controlled, the flowers being kiln-dried under strictly controlled conditions.—per *Mfg. Chem.*, 1941, 236.

Determination of the Pyrethrins.

A number of methods have been published for the determination of the pyrethrins I and II. All suffer from some disadvantages and in each case concordant results can only be obtained by closely adhering to the details of the methods. Owing to the presence of substances such as free chrysanthemic acids, which interfere more or less with the process, results obtained by the different acid methods are not absolutely comparable. In all cases the pyrethrum flowers are extracted with petroleum ether. The assay of kerosene preparations, such as fly-sprays, which may contain perfumes, is never satisfactory due to interfering substances present as impurities or as components of the perfumes used.

The pyrethrins I and II may be determined either separately or combined. It is generally stated that pyrethrin I is more toxic to insects than pyrethrin II and that they are present in about equal proportions, but this is not always so. The process given as a standard in the *B.P.C.* 1934 determines the pyrethrin I only and is the best and simplest for this purpose.

Determination of Total Pyrethrins I and II. This process depends upon the reducing power of the pyrethrins on alkaline copper solution; it is a modification of the method of Folin. The temperatures and times given in the method must be very strictly adhered to and a bulb tube of the correct size must be used. Complete details of the process and a table showing the comparison between the reducing power of the pyrethrins and dextrose, which is used as a standard, are described.—C. B. Gnadinger and C. S. Corl, *J. Amer. chem. Soc.*, 1929, 3054.

Determination of Pyrethrins I and II Separately. The pyrethrins I and II are converted to the chrysanthemum acids by saponification and liberation with acid. The volatile monocarboxylic acid which is formed from the pyrethrin I is distilled off and estimated by titration. The residue from the distillation containing the dicarboxylic acid derived from the pyrethrin II is extracted and determined separately by titration.—H. A. Seil, *Soap*, 1934, 10, 89. A modification of this process.—Ripert, *Ann. Falsif.*, 1934, 25, 580 and 595.

Pyrethrins I and II may be determined quantitatively by hydrogenation with palladium as catalyst yielding hexahydropyrene and the acids of pyrethrins I and II. The carboxylic acid of I is separated by steam distillation and titrated with alkali, and the dicarboxylic acid of II is extracted from the residual filtrate

with ether and subsequently titrated.—F. B. La Forge and F. Aoree, *Soap and Sanitary Chemicals*, 1941, 17, No. 1, 95.

Determination of Pyrethrin II. Pyrethrin II being a methyl ester, yields methyl iodide on refluxing with hydriodic acid. The methyl iodide is estimated volumetrically by converting it into iodic acid, liberating the iodine by means of potassium iodide and titrating with sodium thiosulphate. This method gives results which are lower than those obtained by the acid method.—H. L. Haller and F. Acree, *Industr. Engng Chem., anal. Edn.*, 1935, 343.

Determination of Pyrethrin I in Insecticides. The Wilcoxon method has been adapted to the determination of pyrethrin I in sprays containing essential oils, derris resins, tobacco extract, etc.—D. A. Holaday, *Industr. Engng Chem., anal. Edn.*, 1938, 5.

Freshly-ground pyrethrum flowers, in containers of different kinds, lose from 30 to 43.6% of pyrethrins within a year. Powdered and ground flowers in closed tins continue to lose pyrethrins for more than 2 years but not so rapidly. Storage under suitable conditions is therefore important.—C. B. Gnadinger and C. S. Corl, *Industr. Engng Chem.*, 1932, 901.

Fly Sprays. An efficient fly spray should contain not less than 0.09% of total pyrethrin. Killing-power is increased by the use of a wetting-agent such as coconut oil soap or diglycololeate.—*Pharm. J.*, i/1933, 129.

Evaluation of the Toxicity of Pyrethrum Insecticides. Chemical analyses of pyrethrum extracts proving unreliable for this purpose biological methods are used. Generally they follow the same lines, viz. (1) spraying a definite amount of insecticide of a known concentration at a controlled pressure; (2) recording the time when the insects become paralysed; (3) ascertaining the number of recoveries by setting the insects aside for a definite time. It is preferable to adjust the concentrations so as to obtain a "kill" varying between 40 and 60%.

The National Association of Insecticide and Disinfectant Manufacturers in the U.S.A. have adopted an Official Control Insecticide for purposes of standardisation. Tests are conducted in the Peet and Grady method and the standard error of the mean difference between the average kill and the O.C.I. must be less than three. An example of the method of calculation is given. The mean difference is utilised for grading insecticides.—W. E. Edmonton, *Pharm. J.*, ii/1939, 289. See also F. P. Mackie and H. S. Crabtree, *Lancet*, ii/1938.

PYROXYLINUM

Pyroxylinum (B.P. Add. I). The substance dried at 100° for one hour contains 11.5 to 12.3% of nitrogen. To determine the nitrogen content, a weighed portion of the dried substance is transferred with nitrogen-free sulphuric acid to a mercury-filled nitrometer and shaken, and the liberated nitric oxide measured. Kinematic viscosity at 20° of a 3% w/v solution of the dry substance in acetone, not less than 370 centistokes. Pyroxylinum, *U.S.P. XII*, saturated with alcohol in a dish placed in cold water, ignited at the top and afterwards heated to redness, leaves not more than 0.3% of ash. Yields not more than 0.3% of water-soluble substances.

RHEUM

Rheum (B.P. Add. I). Rhubarb should contain not more than 2% of foreign organic matter. Alcohol (45%) soluble extractive, not less than 35%. Acid-insoluble ash, not more than 1%. A limit test for rhapontic rhubarb is included when on examination in screened ultra-violet radiation with a lens it shows no shining

violet points. Rheum, *U.S.P. XII*, yields not less than 30% of diluted alcohol-soluble extractive; Rheum rhaponticum is excluded.

Rhizoma Rhei, *P. Helv. V*, yields not more than 13% of total ash and not more than 1% of acid-insoluble ash; it contains from 4.23 to 6.56% of free and combined anthracene derivatives calculated as chrysophanic acid, and complies with the following test for the absence of rhapontic rhubarb:—Boil 10 g. of powdered rhubarb with 50 ml. of dilute alcohol for 15 minutes; filter and evaporate the filtrate to 10 ml.; allow to cool and treat with 15 ml. of ether; after 24 hours no crystals should be deposited which when dried and treated with concentrated sulphuric acid produce a purple-red colour.

The method of *P. Helv. V*, for the assay of rhubarb is based upon false assumptions and is totally unnecessary.—L. Rosenthaler, per *J. Amer. pharm. Ass. Abstr.*, 1936, 42.

Fluorescence Test for Rhapontic Rhubarb. On exposure to ultra-violet light rhapontic rhubarb exhibits a violet fluorescence, while genuine Chinese rhubarbs give a velvety brown fluorescence.

Using genuine Chinese rhubarb as a standard for comparison, one can easily detect as little as 1% of added rhapontic rhubarb, using paper strips soaked in a tincture made from the drug. The whole operation must be conducted in a dark room. The wide variation in the intensities of the fluorescence of different samples of rhapontic rhubarb makes it very difficult to carry out exact quantitative determinations by this method.—T. E. Wallis and E. R. Withell, *Quart. J. Pharm.*, 1934, 574.

The presence of the rhapontic variety in galenical preparations can be detected by the fluorescence test. A pledget of cotton or other form of cellulose is immersed in a tincture or other galenical containing rhapontic rhubarb, and then washed in water. It gives a bright blue fluorescence in screened ultra-violet light (wave-length 310—390 μ).—S. K. Crews, *Quart. J. Pharm.*, 1936, 434.

Evaluation on chrysophanic acid content, the results not being absolute, but well adapted for comparative purposes.—A. Tschirch and P. Schmitz, *Quart. J. Pharm.*, 1929, 463.

In the *B.P.* formulæ for pills, vegetable drugs such as aloes, ginger, etc., are taken in "fine powder," and it may be assumed therefore that Pulv. Asafoet. is not sanctioned in Pil. Aloes et Asafoet., or Pulv. Myrrh. in Pil. Rhei Co. Since both these powders are well known in commerce it would have been helpful if the monographs on these and other vegetable drugs had provided the user with information as to which drugs were official in powder form.—*Pharm. J.*, ii/1932, 147.

SACCHARINUM

Saccharinum (B.P.C.). $C_7H_5O_3NS = 183.1$. Determined by the *B.P.* method for Saccharinum Solubile, it contains not less than 97% of the pure substance. M.p., not below 225°. Saccharinum, *U.S.P. XII*, leaves not more than 0.2% of ash, and complies with tests for glucose and lactose, benzoic or salicylic acid and ammonium compounds; 0.2 g. complies with a test for carbonisable substances.

Official qualitative and quantitative methods for the determination of saccharin in foods are described in *Methods of Analysis (A.O.A.C., 1940, 458)*.

Detection in Foodstuffs. To 25 g. of the sample add sufficient boiling water to produce 75 ml. and after one hour, with occasional shaking, acidify with 3 ml. of glacial acetic acid, and add 5 ml. of 20% neutral lead acetate solution. Dilute the mixture to 100 ml., allow to stand for 20 minutes, filter and acidify an aliquot portion (60 ml.) with 5 ml. of hydrochloric acid. Extract the mixture with equal parts of ether and light petroleum (b.p. 30° to 60°), wash the ethereal solution with 5 ml. of water and allow the solvent to evaporate at room temperature. Heat the residue with 5 ml. of a solution of phenol in an equal weight of sulphuric acid at 135° to 140° for 2 hours, dissolve the product in a little hot water and pour into about 250 ml. of cold water. After 3 hours, filter, make alkaline with 10% sodium hydroxide solution and dilute to 500 ml. A magenta or reddish purple colour indicates the presence of saccharin in the original sample. Vanillin if present must be removed by repeated extraction of the ethereal extract with carbon tetrachloride.—W. F. Reindollar, *J. Ass. off. agric. Chem., Wash.*, 1940, 23, 288.

Saccharinum Solubile (B.P.). $C_7H_4O_3NSNa \cdot 2H_2O = 241 \cdot 1$. Contains not less than 98% of the pure substance. M.p. of the washed and dried separated saccharin, not lower than 226°. Complies with tests for absence of benzoate and salicylate and limit of *p*-sulphaminobenzoic acid. Assayed by the B.P. '32 process: boil 0.7 g. with 10 ml. of 30% sodium hydroxide solution for 2 minutes; cool and reflux with 15 ml. of hydrochloric acid for 50 minutes; cool, rinse condenser with 50 ml. of water, and remove acid vapours with a current of air; distil the ammonia liberated on addition of 20 ml. of 30% sodium hydroxide solution into excess N/10 sulphuric acid and back titrate with N/10 sodium hydroxide to methyl red. Saccharinum Sodicum, U.S.P. XII, complies with tests for neutrality, benzoate or salicylate, ammonium compounds, etc.

Tabellæ Saccharini Sodici (U.S.P. XII). Contain 95 to 110% of the labelled amount of $C_7H_4O_3NSNa \cdot 2H_2O$, including all tolerances. Complies with a limit test for ammonium salts. Assayed by refluxing for 1 hour a weighed quantity of powdered tablets with water-hydrochloric acid mixture (40 : 25), followed by an ammonia distillation.

The sweetness of canned fruits in syrup of 30° Brix may be increased to the equivalent of 38° Brix, or about eight degrees of a 38° syrup may be replaced by saccharin without producing foreign flavours. The apparent sweetness of soluble saccharin in syrup of 30° is about 300 to 400 times that of sucrose. Saccharin has little effect on the internal corrosion of cans.—per *Analyst*, 1941, 464.

Dulcin. Dulcin may be used to increase the sweetness of canned fruits from the equivalent of at least 30° to at least 40° syrup, or to replace about 10° of a 30° syrup. The addition of greater amounts produces foreign flavours. The apparent sweetness of dulcin in a syrup of about 30° Brix is about 300 times that of sucrose. On the whole, dulcin is probably less toxic and gives better results than saccharin in canned fruits.—per *Analyst*, 1941, 464.

Determination. About 0.4 g. of sample is rinsed into a Kjeldahl flask with 25 ml. of 18N sulphuric acid. A small quantity of pumice powder is added, and the mixture gently boiled for three hours under a reflux air condenser. When cool, water is carefully added and the contents of the flask made alkaline with 30% sodium hydroxide solution. The alkaline solution is steam-distilled, the ammonia evolved is collected in 40 ml. of N/10 sulphuric acid and the excess of acid estimated. A blank determination is done and the result calculated on the difference of the readings. Each ml. of N/10 sulphuric acid is equivalent to 0.018 g. of dulcin. Results obtained by this method were consistent but high, averaging $103.5 \pm 0.5\%$ and the authors are unable to explain this figure satisfactorily.—J. F. Hirst, F. Holmes, and G. W. G. MacLennan, *Analyst*, 1941, 450.

Colorimetric Determination. Dulcin can be hydrolysed quantitatively to *p*-phenetidine by boiling with 3N sulphuric acid for about 4 hours. By

diazotising the *p*-phenetidine and forming an azo dye with phenol in alkaline solution, dulcin can be estimated. The resulting colour is matched against similar solutions containing known amounts of *p*-phenetidine hydrochloride, and the result is converted to dulcin by multiplying by 1.037. Saccharin has no appreciable effect on the result, and neither has ammonia, which may be produced during the hydrolysis. Sugar and similar substances interfere with the process and composite articles containing these are first extracted with organic solvents to remove essential oils, etc., and then shaken with ethyl acetate to remove the dulcin.—J. Longwell and C. S. Bass, *Analyst*, 1942, 15.

Determination in Beverages. Twenty-five ml. of the sample, or of the corresponding extract after distillation of an alcoholic beverage, are extracted with light petroleum. The petroleum extract is washed with 5 ml. of water and rejected, and the washings are added to the main solution. The dulcin is extracted from this solution with three 30-ml. portions of ethyl acetate, and the united extracts washed with 10 ml. of water. The ethyl acetate is distilled off and the residual dulcin dissolved in 5 to 10 ml. of acetone and rinsed in a 10-ml. Kjeldahl flask. After evaporation of the acetone, 5 ml. of 18N sulphuric acid are added, and the solution is boiled under a reflux air condenser for three hours. It is then cooled, diluted, made alkaline with 15 ml. of 30% sodium hydroxide solution and steam-distilled. The ammonia evolved is trapped in 25 ml. of water and the distillation continued until about 180 ml. have been collected. The distillate is made up to 200 ml.; 40 ml. of this solution are Nesslerised in a 50-ml. tube, and the colour is compared with that developed in equal volumes of water containing 1.5, 2.0 and 2.5 ml. of standard ammonium chloride solution. A blank determination is made at the same time. The standard solution contains 0.2125 g. of ammonium chloride per litre (1 ml. is equivalent to 0.0007145 g. of dulcin).—J. F. Hirst, F. Holmes, and G. W. G. MacLennan, *Analyst*, 1941, 451.

Detection in Saccharin. 100 mg. of the sample and 100 mg. of resorcinol are heated for 1½ to 2 minutes at exactly 180° in 2 ml. of sulphuric acid. The red liquid is cooled, diluted with 5 ml. of water and made alkaline with sodium hydroxide; it then has an orange-red colour and green fluorescence due to saccharin. On oxidation (with an alcoholic solution of iodine, bromine or silver nitrate, etc.) and dilution with 100 ml. of water a violet colour is produced if dulcin is present in the saccharin to the extent of 1%.—H. J. Vlezenbeek, *Pharm. Weekbl.*, 1937, 127.

SANTONICA

Santonica (B.P.C.). Contains not less than 2% of santonin. Assayed by maceration with chloroform, filtration, recovering most of the chloroform from a portion of the filtrate, and with addition of 1.2% baryta, heating off the remaining chloroform; extraction of the filtered and washed acidified liquid is effected with chloroform; the residue after evaporation of the solvent is dissolved in dehydrated alcohol, diluted with hot water to 15% *w/w* of alcohol and filtered; after 24 hours the separated santonin is collected on a tared filter, washed with 15% *w/w* alcohol and dried; a correction for loss due to solubility is allowed.

Flores Cinæ, *P.G. VI*, by the process prescribed, yields not less than 2% of santonin. Flos Cinæ, *P. Helv. V*, is required to show a santonin content of not less than 1.8%. Assayed by the following process:—Shake 10 g. for ½ hour with 100 g. of benzene, and filter; recover the benzene from 81 g. of filtrate (= 8 g. of drug), removing the last traces in an oven at 103° to 105°, cool, dissolve the residue by refluxing for 15 minutes with 40 ml. of 15% (*w/w*) alcohol. Filter through cotton wool, warm and wash flask and funnel twice with 5 ml. of 15% alcohol. Again cool, add 0.1 g. of

kaolin and reflux a second time for 15 minutes and filter hot through a 6 cm. diam. filter, washing with 2 portions of 5 ml. of 15% alcohol. Set aside in the dark for 24 hours, weigh the solution and filter, washing three times with 2 ml. of water. Dry the filter for 1 hour at 103° to 105°, and dissolve the crystals with 2 portions of 5 ml. of chloroform. Evaporate, dry for 1 hour at 103° to 105° and weigh. For each g. of solution from which the santonin was crystallised add a correction of 0.0006 g. The assay process of the *P.G. VI* is similar.

Assay. Santonin in wormseed cannot be determined as the 2:4-dinitrophenylhydrazine since other constituents interfere, giving a high result. The following method may be used:—The powdered drug is moistened with ammonia, dried, and extracted with benzene. The residue, after evaporating the benzene, is digested on a water-bath with a saturated solution of barium hydroxide, and the liquid filtered, acidified with hydrochloric acid and set aside for 24 hours to allow the santonin to crystallise. The crystalline residue is collected on a sintered glass crucible, washed with a little water, dried at 100° and weighed.—M. M. Janot and M. Mouton, *Bull. Sci. pharm.*, 1936, 43, 708.

Santoninum (B.P.). $C_{18}H_{18}O_3 = 246.1$. M.p., 171° to 174°. Ash, not more than 0.1%. 0.1 g. with 2 ml. of sulphuric acid is not darker than pale brown, and on dissolving forms a clear solution not darker than yellow. The alcoholic solution should be neutral to litmus. Santoninum, *N.F. VII*, leaves not more than 0.1% of ash. 0.5 g. boiled with 20 ml. of water and 2 ml. of dilute sulphuric acid, cooled and filtered, produces no cloudiness with Mayer's reagent or with iodine solution, even after standing for 3 hours; 0.1 g. complies with the specified test for carbonisable substances. Santonine, *Fr. Cx.* 1937, melts at 170°, α_D (2% solution in chloroform) -172.26° .

Official methods for the determination of santonin in mixtures are described in *Methods of Analysis (A.O.A.C., 1940, 614)*. The methods are a modified Langer's method and the dinitrophenylhydrazine method. A tentative method for the determination of santonin in santonica is also described (*ibid.*, 615).

SAPONES

Sapo Animalis (B.P.). Curd soap loses at 110°, 20 to 30%. In powder, loss at 110°, not more than 5%. Solidifying-point of the fatty acids, not less than 42°. Limit tests for alkali hydroxide, and free fatty acids, alkali carbonate and free fat are described. The *B.P. Add. I* raises the limit of free fat to 0.8%, and does not require the previously stated amount of 20 g. to be used for preparation of the fatty acids for the solidifying-point. Sodii Stearas, *U.S.P. XII*, complies with tests for alcohol-insoluble substances and free fatty acids; solidifying-point of the free fatty acids, not below 54°; loss, on evaporating at 80° a mixture of the substance and dried sand with alcohol and drying at 110°, not more than 5%.

Sapo Durus (B.P. Add. III). The sodium soap of suitable oil or oils or of fatty acids derived therefrom, other than coconut oil or palm kernel oil or their fatty acids. After being reduced to

thin shavings, or powdered, it complies with tests for limits of alkali hydroxide, free fatty acid and total free alkali described for Sapo Mollis. Unsaponified neutral fat and unsaponifiable matter, not more than 1%; chloride and other alcohol-insoluble substances, not more than 1%. A test for absence of resin is included. The separated fatty acids have the following characters:—Solidifying-point, not less than 28° (unless made entirely from olive oil); acid value, not more than 205. Loss at 100°, 20 to 30%. *Hard soap in powder* of the following higher limits is allowed:—alkali hydroxide increased by 30%, free fatty acid by 25%, total free alkali by 30%, unsaponified neutral fat and unsaponifiable matter, and chloride and other alcohol-insoluble substances by 20%. Loss at 110°, not more than 5%. Sapo Durus, *U.S.P. XII*, dissolved in alcohol, evaporated with sand, and dried at 110°, loses not more than 36%, or 10% for powdered soap. The dried soap yields not more than 1% of alcohol-insoluble matter and not more than 0.15% of water-insoluble substances. The fatty acids have a solidifying-point of 18° to 23°, an acid value of 185 to 205, and an iodine value of 83 to 92.

The *B.P.* figure of 0.5% for free fat in soaps is too low. The limit for Sapo Durus should be raised to 1%, for Sapo Mollis to 0.7%, and for Sapo Animalis to 0.7 to 0.8%.—Report of Sub-Committee, *Analyst*, 1934, 104.

Castile Soap (Jabón Castilla). By a Spanish Royal Order (Dec. 14, 1927) the name must apply only to a soap prepared in Spain, in the manufacture of which no fats other than olive oil have been used: containing not more than 2% chlorides (as sodium chloride): maximum water content 25%; maximum free alkali content 0.3%. The soap must be white and must be soluble in water or alcohol without residue.—*Pharm. J.*, i/1928, 164.

A method of determining the detergent action of soaps depends on the quantity of carbon which the soap solution will carry through filter paper. The "**carbon number**" is the number of grammes carried by 1 kg. of solution under standard conditions.—J. W. McBain and co-workers, *J. Soc. chem. Ind., Lond.*, 1923, 273.

Sapo Kalinus (*B.P.C.*). Potash soap yields, by the *B.P.* method for Sapo Mollis, not less than 44% of fatty acids of linseed oil, having an iodine value of 179 to 210. Complies with limit tests for chlorides and other alcohol-insoluble substances, alkali hydroxide, alkali carbonate, and free fatty acid.

Sapo Mollis (*B.P. Add. III*). Includes sodium or potassium soaps of a suitable vegetable oil or oils or the fatty acids therefrom, other than coconut oil, palm kernel oil or their fatty acids. Contains not less than 44% of fatty acids. Alkali hydroxide in 10 g. determined by titration of a solution in neutral alcohol after the addition of barium chloride solution, equivalent to not more than 1 ml. of N/10 hydrochloric acid. Free fatty acid in 10 g., equivalent to not more than 0.2 ml. N/10 sodium hydroxide. Total alkali in 10 g., equivalent to not more than 1 ml. N/10 sulphuric acid. Unsaponified neutral fat and unsaponifiable matter, not more than 0.8%; chlorides and other alcohol insoluble substances, not more than 3%. The separated fatty acids have an acid value of not more than 205, iodine value of not less than 83 and solidifying-point not higher than 31°. A test for absence

of resin is included. Fatty acids are determined by extraction of an acid solution with ether, washing the ether extracts with water, removal of the ether and drying the residue at 80°. Sapo Mollis Medicinalis, *U.S.P. XII*, is made from vegetable oils excluding coconut oil and palm kernel oil. Contains not more than 52% of moisture, determined by the toluene distillation method. Alcohol-insoluble matter, not more than 3%. Free alcohol-soluble alkali, calculated as KOH, not more than 0.25%; the fatty acids have an acid value of not more than 205 and iodine value, not less than 85. Sapo kalinus, *P.G. VI*, contains not less than 40% of the fatty acids of linseed oil.

Spiritus Saponatus (*B.P.C. Supp. II*). Determined by the process for Sapo Mollis, *B.P. Add. III*, yields not less than 27% *w/v* of fatty acids, which comply with the requirements described for the fatty acids of Sapo Mollis. Alcohol content, 28 to 31% *v/v* of ethyl alcohol.

Soft Soaps for Insecticides. The specification of the Association of British Insecticide Manufacturers (Bulletin No. 82: Ministry of Agriculture and Fisheries) requires soft soap for spraying purposes to dissolve completely in distilled water to a clear solution, to contain not more than 1% of free caustic alkali (KOH) or 3% of free alkali carbonate (K_2CO_3); not less than 95% of the total alkali, expressed as K_2O , to be K_2O . Percentage of fatty and resin acids to be declared.

Analysis of Soap. Methods of soap analysis have been under investigation by the Sub-Committee on Methods of Soap Analysis of the Analytical Methods Committee of the Society of Public Analysts. Reports describing recommended processes for carrying out the following determinations on soap have been issued by the Sub-Committee as follows:—No. 2, Unsaponifiable Matter (*Analyst*, 1935, 537); No. 3, Free Alkali (*ibid.*, 1937, 36); No. 4, Free Alkali and Silica in Silicated Soaps (*ibid.*, 1937, 865); No. 5, Rosin (*ibid.*, 1937, 868).

Detection of Soap Stains. The iron hydronamate test for carboxylic acids is a sensitive test for stains from commercial soaps in cases of criminal abortion. The stains on fabrics may be compared qualitatively in ultra-violet light.—M. W. Partridge, *Quart. J. Pharm.*, 1942, 21.

Triethanolamine. **Determination in Emulsions.** Mix about 5 g. of emulsion with 20 ml. of water, heat on a water-bath for 5 minutes, add sulphuric acid until a blue colour is given with congo-red paper and heat for another minute adding acid if necessary. Cool and extract with two 25 ml. quantities of ether. Shake the ethereal extract with 20 ml. of approximately N/10 sulphuric acid and distil off the ether. Re-extract the aqueous layer with ether and add it to the aqueous residue from the previous extraction. Heat on a water-bath to free from ether and precipitate the sulphuric acid with barium hydroxide solution. Warm the mixture, centrifuge, pour off the clear liquid, wash the residue and precipitate the excess of barium from the boiling liquid with carbon dioxide. Cool, filter, concentrate under reduced pressure to 3 ml. and wash the residue into a 50 ml. beaker with 15 ml. of ethyl alcohol through a Hirsch funnel, evaporate the alcohol and add 3 ml. of hydrochloric acid. Evaporate the excess of acid, collect the hydrochloride, dry in a steam-oven for 30 minutes and cool in a desiccator. Wash on to a sintered glass crucible with 4 quantities each of 5 ml. of pure isopropyl alcohol. Dry to constant weight at 100°. Weight of triethanolamine hydrochloride $\times 0.803$ gives the weight of triethanolamine. To allow for the solubility of triethanolamine in isopropyl alcohol add 0.003 g. to the ascertained weight of hydrochloride. The method can be applied with minor modifications to emulsions containing sulphonated alcohols or various other substances.—C. J. Eastland, N. Evers and T. F. West, *Analyst*, 1937, 261.

Identification of some Amine Emulsifiers. Several of the commonly used amino compounds used to replace soaps in cosmetics and similar preparations may be identified by the melting points of their sulphonyl or oxalate derivatives. Triethanolamine and tripropanolamine do not give crystallisable derivatives with these reagents.—I. S. Shupe, *J. Ass. off. agric. Chem., Wash.*, 1942, 25, 227.

SCILLA

Scilla (*B.P.* and *B.P. Add. IV*). Ash, not more than 6%. No biological assay is included and no standard is recommended by the Permanent Commission on Biological Standardisation of the League of Nations, since the biological assay of squill estimates the cardiac glycosides present, which are not the constituents responsible for its expectorant effect for which it is chiefly used in medicine. The *B.P. Add. IV* allows the use of Indian squill. The *N.F. VII* allows the use of *Urginea maritima* (Linné) Baker or of Indian squill, and requires the drug to contain not more than 2% of foreign organic matter and not more than 2% of acid-insoluble ash.

The *U.S.P. X* included the following biological requirement:—“Tincture of Squill injected into the ventral lymph sac of a frog has a minimum systolic dose of not less than 0.0055 c.c. and not more than 0.0065 c.c.; equivalent to not less than 0.00000046 g. and not more than 0.00000054 g. of ouabain for each g. of body weight of frog.” Thus ouabain was prescribed as a standard for squill and 100 ml. of a tincture was required to be equivalent to 8.3 mg. of ouabain. Ouabain is, however, an unsuitable standard for squill if different methods of assay are to be permitted, since the relative potency of squill and ouabain differs according to the species (see Burn, *Pharm. J.*, i/1927, 328).

Acetum Scillæ *B.P. Add. VI*. Contains active constituents equivalent to 10% *w/v* of squill. Sp. gr., 1.02 to 1.035; acidity of 10 ml. equivalent to 9.0 to 10.5 ml. of *N/1* sodium hydroxide, using phenolphthalein indicator.

Oxymel Scillæ (*B.P.*). Sp. gr., about 1.27; α_D of 25% *w/v* aqueous solution, using decolourising charcoal if necessary, + 0.6° to + 1.9°. Acidity of 20 ml. diluted with 20 ml. water, 8.0 to 9.0 of *N/1* sodium hydroxide to phenolphthalein indicator.

Galenical preparations develop a levorotation on standing, possibly due to hydrolysis of the squill glycosides. The average rotation of the tincture (*B.P. '32*) is -2.0°, and of the vinegar, -3.5°. —L. McGraham, *Analyst*, 1937, 539.

Scillaren is a crystalline glycoside of squill which could be used as a standard for biological estimations of squill if it were not a proprietary preparation. The average potency of tincture of squill *B.P. '32*, is equivalent to that of a 0.013% solution of Scillaren.

SENNA

Sennæ Folium (*B.P.*). Contains not more than 1% of stalks and not more than 2% of other foreign organic matter. Ash limit, 12%. Acid-insoluble ash limit, 3%. Senna, *U.S.P. XII*, contains not more than 8% of stems and not more than 2% of pods or other foreign organic matter. Acid-insoluble ash, not more than 3%. **Folium Sennæ**, *P. Helv. V*, consists only of the leaflets of *Cassia angustifolia* (Tinnevely senna), and may give up to 12% of ash.

Sennæ Fructus (*B.P.*). Contains not more than 2% of foreign organic matter. **Fructus Sennæ**, *P. Helv. V*, is the dried fruit of *Cassia acutifolia* and of *Cassia angustifolia*, and should yield not more than 6% of ash.

The standard is expressed in a very exceptional manner, and allows the presence of 2% of any other pods or even of dirt. Many of these new standards for crude drugs appear to be based on the *U.S.P. X*, but are unsatisfactory because they do not discriminate between harmless admixture and dangerous foreign organic matter.—*Pharm. J.*, ii/1932, 186.

Ether shaken with a slightly acidified senna extract gives the Bornträger reaction—a pink or red colour with ammonia water. If the ether be shaken with a saturated solution of nickel acetate, the aqueous layer turns red, and if this be separated and potassium hydroxide added a violet precipitate forms, which is stated to be a characteristic test for senna.—*U.S.D.*, 1926.

SINAPIS

Black Mustard contains the glycoside sinigrin, i.e., potassium myronate, $C_{10}H_{15}KNS_2O_9$, with myrosin, which is similar to the ferment emulsin in bitter almonds. This glycoside splits up under the influence of water with evolution of allyl isothiocyanate— $C_3H_5NCS=99.112$, the principal constituent of the essential oil—potassium acid sulphate and glucose. *Sinapis Nigra*, *U.S.P. XII*, contains not more than 5% of other seeds or foreign organic matter; yields not less than 0.6% of allyl isothiocyanate; assayed by maceration with water for 2 hours at 37°, addition of alcohol and distillation into a mixture of solution of ammonia and N/10 silver nitrate; after allowing this mixture to stand overnight it is heated in a boiling water-bath, cooled, adjusted to volume and filtered; an aliquot part is then acidified with nitric acid and titrated with ammonium thiocyanate. *P.G. VI* and the *Fr. Cx.* 1937 require black mustard seeds to yield at least 0.7% of the allyl compound.

White Mustard Seeds (*Sinapis alba*) do not yield allyl mustard oil, but acrinyl isothiocyanate, $C_6H_4OH \cdot CH_2O \cdot NCS$. The **sinalbin** contained in the seeds under the influence of myrosin and water decomposes forming that oil, sinapin acid sulphate, and glucose. The oil in question has a sharp taste. It decomposes on heating. It is insoluble in water, but readily in alcohol or ether. As the black seeds contain an excess of their glycoside and the white an excess of the ferment the combination of the two produces the strongest effect.

Some work by Prof. Greenish, however (*Pharm. J.*, i/1912, 203), shows that in all the samples of black mustard seed examined—both old and new—there was sufficient myrosin to decompose all the sinigrin present, and that properly preserved black mustard seeds retain their myrosin unimpaired for many years. Two samples examined contained sufficient myrosin to decompose a much larger quantity of sinigrin than the seeds themselves contained.

J. Gadamer has made a close study of these various constituents—see Schmidt, *Pharm. Chem.*, Vol. II, Sections 1 and 2.

The following definitions were given by the Food and Drug Administration of the U.S. Dept. of Agriculture:—Mustard seed as the seed of *Sinapis alba* L. (white mustard), *Brassica nigra* (L.) Koch (black mustard), *B. juncea* (L.) Cosson, or varieties or related species of the two latter. *Sinapis alba* contains no appreciable amount of volatile oil: contains not more than 5% of total ash and not more than 1.5% of ash insoluble in hydrochloric acid. *Brassica nigra* and *B. juncea* yield 0.6% of volatile mustard oil (calculated as allyl isothiocyanate): varieties and species of these two yield not less than 0.6% of volatile mustard oil of similar character and composition. These mustard seeds yield

not more than 5% of total ash, nor more than 1.5% of ash insoluble in hydrochloric acid. GROUND MUSTARD SEED, mustard meal; unbolted ground mustard seed, conforming to standards for mustard seed. MUSTARD CAKE is ground mustard seed minus a portion of the fixed oil. MUSTARD FLOUR, ground mustard, "mustard": the powder made from mustard seed with the hulls largely removed and with or without removal of portion of fixed oil: contains not more than 1.5% of starch, nor more than 6% of total ash. PREPARED MUSTARD: a paste composed of a mixture of ground mustard seed and/or mustard flour and/or mustard cake, with salt, a vinegar, with or without sugar and/or dextrose, spices or other condiments. In the fat-, salt-, and sugar-free solids, it contains not more than 24% of carbohydrates, not more than 12% of crude fibre, nor less than 5.6% of nitrogen, the carbohydrates being calculated as starch.—S.R.A., F.D. No. 2 Rev. 5, Nov. 1936.

Oleum Sinapis Expressum. *Detection of Linseed Oil in Mustard-seed Oil.* A micro-bromide test is described as follows: A drop of oil (0.04 to 0.06 g.) is spread on a microscope slide (which must be free from grease) by drawing it out with the narrow edge of a second slide along the whole length of the first slide. The film so obtained is exposed for 20 to 25 minutes in an atmosphere of bromine and then left in the air for a short time to remove excess of bromine. Linseed oil yields a hard, colourless, gritty film with wrinkled surface. Mustard-seed oil gives a soft, opaque and glossy film without tendency to wrinkle. The slide is now placed horizontally on the edge of a small rectangular trough and covered with a mixture of equal parts of ether and petroleum ether and the film stirred with a very thin glass rod and examined for the formation of a flocculent precipitate. Mustard-seed, cottonseed, arachis, kapok and mowrah oils give no precipitate. Linseed oil 5% and upwards in mustard-seed oil gives a precipitate.—S. Neogi, *Analyst*, 1935, 91.

Detection and Determination of Adulteration. The method depends on the presence of erucic acid, the lead salt of which is sparingly soluble in cold alcohol and separates with the solid fraction of the fatty acids. The figure determined is the percentage of iodine absorption of the solid fraction separated under standard conditions and expressed in terms of erucic acid.

From 500 to 510 mg. of the oil is accurately weighed in an Erlenmeyer flask and saponified with 5 ml. of alcoholic potash (40 ml. of aqueous potassium hydroxide solution of sp. gr. 1.5, 40 ml. of water and alcohol 95% to 1 litre) for 1 hour. The saponified solution is treated with 20 ml. of lead acetate solution (lead acetate 50 g., 96% acetic acid 5 ml., alcohol (80% v/v) to 1 litre), and 3 ml. of water and 1 ml. of 96% acetic acid, and heated under a reflux condenser until the lead salts are dissolved. The solution is cooled slowly to room temperature, kept in an incubator at 20° for 14 hours and is then transferred to a 3G/10 sintered glass crucible with the aid of alcohol 70% (cooled to 20°). The precipitate is washed with 12 ml. (in small portions) of the cooled alcohol previously used for rinsing the flask, and then dissolved in 20 ml. of a hot mixture of equal parts of alcohol 95% and acetic acid 96% and the warm solution and the crucible are washed into a 350 ml. bottle of Jena glass with 10 ml. of alcohol and acetic acid mixture. The iodine value of the lead salt solution is then determined by adding 20 ml. of fresh N/5 alcoholic iodine solution and 200 ml. of water, shaking, and setting aside in the dark for 1 hour and then titrating with N/10 thiosulphate. The "value" calculated is the percentage of iodine absorption in terms of erucic acid. 1 ml. of N/10 thiosulphate corresponds to 16.9 mg. of erucic acid. Examination of a number of authentic oils from various species of *Brassica* and of mixtures of these oils gave results between 42.8 and 49.7. Linseed, sesame, niger-seed and arachis oils all gave results in the neighbourhood of 2, while with castor oil the value found was about 0.2. Figures are also given showing the values obtained with various mixtures of genuine mustard-seed oil and other vegetable oils thus providing a method for the determination of the proportion of adulterant.—Sukumar Neogi, *Analyst*, 1936, 597.

Oleum Sinapis Volatile (B.P.C.). Contains not less than 92% w/w of $C_9H_{17}NCS$. Sp. gr., 1.014 to 1.025; n_{D20} , 1.525 to 1.530. Assayed by heating an alcoholic dilution under a reflux condenser on a water-bath for 30 minutes with N/10 silver nitrate and strong solution of ammonia, followed by adjustment to volume, filtration and titration of an aliquot part with N/10

ammonium thiocyanate. Oleum Sinapis Volatile, *U.S.P. XI*, is renamed Allylis Isothiocyanas in the *U.S.P. XII* and may be the natural or synthetic product. It contains not less than 93% w/v of C_3H_5NCS . Sp. gr. at 25° , 1.013 to 1.020; n_{D20° , 1.5275 to 1.5310. Distills completely between 148° and 154° , the first and last 10% of the distillate having almost the same sp. gr. as the original oil.

Determination of essential oil of mustard in black mustard.—Miesemaeker and Boivin, *J. pharm. Chim., Paris*, 1930, 122, 478.

Determination of Essential Oil in Mustard Flour. The glycosides are hydrolysed under standard conditions, the phosphate present is precipitated with $MgCO_3$ and NH_3 and the sulphate precipitated in the filtrate with benzidine; the washed benzidine sulphate is then dissolved in NaOH and back titrated with H_2SO_4 .—R. C. Terry and J. W. Corran, *Analyst*, 1939, 164-172.

SODIUM

Notes on other sodium salts are included under the corresponding acids (see Index).

Sodii Bicarbonas (B.P.). $NaHCO_3 = 84.00$. Contains from 99% to the equivalent of 101% of $NaHCO_3$. The *U.S.P. XII* substance, after drying over sulphuric acid, contains not less than 99% of $NaHCO_3$. 1 g. in 20 ml. of distilled water not above 15° should not contain sufficient carbonate to produce a red tint on adding 2 ml. of N/10 hydrochloric acid and 2 drops of phenolphthalein solution. Carbonate Acide de Sodium, *Fr. Cx.* 1937, by ignition to carbonate; contains 99.5% of the pure substance.

The B.P. Limit Test for Carbonate. The test for carbonate in sodium bicarbonate is not sensitive to less than about 4%, but the assay would condemn a sample, if dry, containing 2% of sodium carbonate.—W. H. Linnell, *Pharm. J.*, ii/1932, 531.

The pH of 1% sodium bicarbonate solution is raised from 8.37 to 8.60 by the addition of 2.05% of normal carbonate. In practice, owing to the limited accuracy of the official method, a considerably higher percentage would escape notice. The following is a more sensitive test:—1 g. $NaHCO_3$ is dissolved in CO_2 -free water, exactly 10 ml. of 0.01% phenolphthalein solution is added, and the solution diluted to 100 ml. in a Nessler glass. A comparison solution is prepared by diluting 5 to 10 ml. N/10 NaOH and exactly 0.3 ml. of the same phenolphthalein solution to 100 ml. in a second Nessler glass. The test solution must not be deeper than the comparison solution.—C. Morton, *Pharm. J.*, i/1933, 3.

2 g. of sodium bicarbonate is added to 15 ml. of a saturated solution of sodium chloride and allowed to stand for 2 minutes. Undissolved bicarbonate is filtered off and the filtrate is made up with CO_2 -free water to 25 ml. and treated with 0.1 ml. of 1% phenolphthalein solution. The colour of the liquid is then observed against a white background. The presence of 0.5% of sodium carbonate is shown by a distinct pink colour.—T. Sabolitschka, per *Quart. J. Pharm.*, 1939, 121.

Tabellæ Sodii Bicarbonatis (N.F. VII). Contain 92.5 to 107.5% of labelled amount of sodium bicarbonate, including all tolerances.

Sodii Carbonas (B.P.). $Na_2CO_3 \cdot 10H_2O = 286.15$. Contains from 99% to the equivalent of 102% of the pure substance. The substance of the *Fr. Cx.* 1937 loses not more than 63% on ignition to red heat and, by titration in boiling solution to phenolphthalein, contains not less than 37% of the anhydrous salt.

Sodii Carbonas Exsiccatus (B.P.). $\text{Na}_2\text{CO}_3=106.0$. Loses not more than 2% at 110° , and then contains not less than 99.5% of Na_2CO_3 .

Sodii Carbonas Monohydratus (U.S.P. XII). When dried to constant weight at 110° loses 10–15% and contains not less than 99.5% of Na_2CO_3 .

Sodii Chloras (B.P.C.). $\text{NaClO}_3=106.5$. Contains not less than 99% of NaClO_3 .

Sodii Fluoridum (B.P.C.). $\text{NaF}=42.00$. Contains not less than 90% of NaF . Assayed by titration at 80° of a solution in carbonate-free alkali, in the presence of sufficient neutral sodium chloride to saturate at end of titration, with N/10 potassium aluminium sulphate, using methyl red as indicator.

Sodii Hydroxidum (B.P. Add. I). $\text{NaOH}=40.00$. Contains not less than 95% of total alkali, calculated as NaOH , and not more than 2.5% of Na_2CO_3 ; assayed similarly to the potassium compound. Limit test for chlorides and sulphates are included. The U.S.P. XII substance assayed similarly to the potassium-compound, contains not less than 95% of total alkali as NaOH , of which not more than 3% is Na_2CO_3 .

Liquor Sodii Hydroxidi (B.P. Add. IV). Contains 3.4 to 3.7% w/v of total alkali as NaOH .

British Standard Density-Composition Tables for Aqueous Solutions of Caustic Soda (B.S.S. No. 824—1938), designed for use in conjunction with British Standard Density Hydrometers, give the concentration in g. of NaOH per 100 g. and in g. of NaOH per 1000 ml. corresponding to densities of 1 to 1.530 for each 5° over the range of 10° to 40° .

Detection and Determination of Sodium. Sodium may be detected and determined by means of dihydroxytartaric acid, $[(\text{HO})_2\text{C}(\text{COOH})_2]$. The reagent is used as a freshly prepared solution of its potassium salt prepared by neutralising until slightly pink a solution of 1 g. of the acid in 20 ml. of water containing 1 drop of phenolphthalein solution and cooled in ice, with N/1 KOH , also cooled in ice. The solution should not be kept for more than 1 day. The solution to be tested is cooled below 5° and mixed with an equal volume of the reagent solution. The mixture is allowed to stand below 5° . In the presence of sodium a white crystalline precipitate forms in from 1 minute to several hours according to the proportion present. The test solution should be neutral and contain no other metals except magnesium and the alkali metals. For determining sodium the reagent is prepared by dissolving 1 g. of dihydroxytartaric acid in 5 ml. of water, adding 35 ml. of ice-cold water and 5.5 ml. of M/1 potassium carbonate solution. To 5 ml. of the sodium solution, containing 1.0 to 1.5% of sodium as the chloride, sulphate or nitrate, add the whole of the above quantity of reagent with stirring, maintaining a temperature of 5° . Allow to stand for 5 hours at this temperature, collect the precipitate, wash with 10 ml. of ice-cold water and place precipitate and paper in acidified permanganate solution consisting of 100 ml. of N/10 potassium permanganate, 100 ml. of 5N sulphuric acid, 100 ml. of 6.3N phosphoric acid and 2 ml. of a freshly prepared 0.05% solution of ammonium metavanadate in water acidified with dilute sulphuric acid. Allow to stand at room temperature for 20 minutes, heat to 50° and titrate with N/10 oxalic acid. Each ml. of permanganate used is equivalent to 0.000767 g. of Na. Add to the result 0.0051 g. of Na to correct for the slight solubility of sodium dihydroxytartrate. Alternatively the sodium may be determined gravimetrically by dissolving the precipitate in sulphuric acid, evaporating to dryness and igniting to Na_2SO_4 .

Sodium may be detected and determined by means of zinc uranyl acetate. The solution of the reagent is obtained by mixing a solution of 10 g. of uranyl acetate in 6 g. of acetic acid (30%) and 65 g. of hot water with a solution of 30 g.

of zinc acetate in 3 g. of acetic acid (30%) and 65 g. of hot water. The mixed liquids are maintained at 19° to 21° for several hours and any crystals of the triple salt, $\text{NaZn}(\text{UO}_2)_2 \cdot (\text{C}_2\text{H}_3\text{O}_2)_6 \cdot 6\text{H}_2\text{O}$, are removed by filtration. *Detection of sodium*.—The test solution is neutralised and mixed with an equal volume of the reagent. In the presence of sodium yellow crystals of sodium zinc uranyl acetate are produced. *Determination of sodium*.—To 10 ml. of the neutral solution containing not more than 0.08 g. of sodium is added 100 ml. of the reagent, and the mixture is allowed to stand at 19° to 21° for 30 to 45 minutes with occasional stirring. The crystals are collected, washed with five successive quantities each of 2 ml. of the reagent and then with five successive quantities each of 2 ml. of alcohol saturated at 20° with sodium zinc uranyl acetate. The precipitate is washed finally with ether, dried at 40° for 10 to 15 minutes and weighed. Each gramme of precipitate is equivalent to 0.01495 g. of Na.

STRAMONIUM

Stramonium (B.P.). Contains not more than 2% of foreign organic matter, not more than 20% of stems, not more than 1% of stems greater than 4 mm. in width, and not less than 0.25% of alkaloids of stramonium, calculated as hyoscyamine. Ash limit, 20%. Acid-insoluble ash limit, 4%. Assayed by the B.P. process for *Belladonnæ Folium*.

Stramonium, *U.S.P. XII*, yields not less than 0.25% of alkaloids, not more than 4% of acid-insoluble ash and contains not more than 3% of stems over 8 mm. in diameter. Assayed by the process described for *Hyoscyamus, U.S.P. XII*. *Folium Stramonii, P. Helv. V*, contains not less than 0.2% of alkaloid and may yield up to 21% of ash.

Tentative methods for the determination of alkaloids of stramonium leaf in ointments are described in *Methods of Analysis (A.O.A.C., 1940, 599)*.

STROPHANTHUS

Strophanthus (B.P.). From *Strophanthus kombé*. Foreign organic matter, not more than 2%. Ash, not more than 5%. *Strophanthus, N.F. VII*, assayed on a tincture prepared by the process for Tincture of *Strophanthus, N.F. VII*, in each gramme possesses a potency equivalent to not less than 55.0 mg. of standard ouabain. *Semen Strophanthi, P.G. VI*, consists of the seeds of *Strophanthus gratus* (Wallich and Hooker) Franchet, and contains not less than 4% of anhydrous *g-strophanthin*; assayed by the process given:—Reflux 7 g. of drug with 70 g. of absolute alcohol for 1 hour, adjust to weight and filter. Take 51.5 g. of filtrate (= 5 g. of drug) and distil until 1 or 2 g. remain, add absolute alcohol to 5 g. and then 30 g. of petroleum ether. If there is no deposit within 1 hour, shake forcibly with 2 or 3 drops of alcohol. Decant the alcohol-petroleum ether solution and wash twice with 5 g. of petroleum ether. Warm the precipitate on a water-bath with 10 ml. of water, add and warm with 5 or 6 drops of basic lead acetate solution, filter and wash four times with 5 g. of water. Pass hydrogen sulphide through the warm filtrate and after 2 hours filter, washing 3 times with 5 g. of water. Evaporate the filtrate

to 5 g.; transfer to a cylinder with 2 portions of 1 g. of water and evaporate to 2 to 2.5 g. Allow to crystallise for 24 hours and finally dry at 105° to 110°. Semen *Strophanthi*, *P. Helv. V*, is the seed of *Strophanthus kombé* Oliver.

The geographical range of *S. kombé* is limited. It might be well to order the use of *S. hispidus* instead—it is more easily obtained and is the only other species giving the green colour with sulphuric acid.—E. M. Holmes, *Pharm. J.*, i/1919, 33.

Recent specimens of *S. kombé* are much mixed with *S. Courmontii*, whilst samples of *S. hispidus* can no longer be found unadulterated in commerce, and are entirely, or in large proportion, seeds of *S. sarmentosus*. A useful table gives the characters of *Strophanthus* species found in commercial samples.—F. J. Mathieson, per *Quart. J. Pharm.*, 1928, 260, 262.

Four colour tests are recommended for the identification of the seeds of various species of *Strophanthus*.—E. M. Smelt, *Quart. J. Pharm.*, 1933, 467.

Tinctura Strophanthi (B.P.). Tincture of strophanthus is compared with a standard tincture of strophanthus by one of the biological methods described under *Digitalis Pulverata*, and is diluted to be of the same potency as the standard tincture. This standard is equivalent to a 0.42% solution of the international standard ouabain (crystalline strophanthin-g), or to a 0.33% solution of anhydrous ouabain as determined by a biological comparison made by the frog method. Tinctura *Strophanthi*, *P. Helv. V*, contains from 0.19 to 0.21% of strophanthin determined by conversion to strophanthidin and multiplying by the factor 2.158.

Seeds of *Strophanthus Eminii*. The results now accumulated indicate that the seeds of this species of strophanthus are similar in their pharmacological action to those of *S. kombé*, that the tincture made from them presents no difficulties in biological assay, and that the mixture of glycosidal principles obtained from them is similar in chemical composition and in therapeutic effects to the strophanthin obtained from the seeds of *S. kombé*.—British Pharmacopœia Commission, *Quart. J. Pharm.*, 1935, 61.

***Strophanthus dichotomus* DC.** contains a powerful cardiac poison. The tincture can be differentiated from tinctures prepared from the seven species of *Strophanthus* examined by Smelt (*supra*) by the following tests applied to 1 ml. portions of the residue obtained by evaporation on a water-bath. Sulphuric acid 75%—brownish violet in 10 to 15 minutes; phenol and hydrochloric acid—pink to cloudy bluish-green; resorcinol and hydrochloric acid—pink-brown to red-orange.—A. H. Millard, *Pharm. J.*, ii/1936, 147.

Preparation of *Strophanthin-E*. The crushed seeds of *S. Eminii* were freed from fat by light petroleum and extracted by percolation with alcohol (90%) at laboratory temperature. The percolate was concentrated under reduced pressure and treated with a slight excess of basic lead acetate. After filtration the solution was freed from lead by excess of hydrogen sulphide. The solution was saturated with ammonium sulphate, and the sticky precipitate which formed was extracted with alcohol. The alcoholic solution was either neutralised with a little sodium hydroxide and precipitated with ether or precipitated without neutralisation. The strophanthin was dried at 100° *in vacuo*. The yield is from 5 to 7% of the fat-free seeds, and is similar to that obtained from *S. kombé*.—I. D. Lamb and S. Smith, *Quart. J. Pharm.*, 1935, 71.

Chemical Assay. Haycock's Method. The powdered seeds (20 g.) are freed from oil with petroleum ether and exhausted with alcohol 70%. This tincture is evaporated at a low temperature, dissolved in 100 ml. of water, filtered, 3.2 ml. of sulphuric acid (25%) added, then shaken out thrice with 20 ml. of ether. The aqueous acid solution is warmed for 1 hour at not exceeding 75°. This decomposes the strophanthin present into strophanthidin and strophanthobiose methyl ether. It is then cooled and shaken out with 10 ml. of chloroform. This is evaporated to a low bulk, allowed to crystallise out and dried below 65°. The result divided by the factor 0.365 gives the amount of

strophanthin present. Various samples of the seed by this method gave 3.1 to 4.57% strophanthin. A standard of 0.1% w/v strophanthin is suggested.—J. Haycock, *Pharm. J.*, i/1911, 553.

FROMME'S 1910 ASSAY METHOD. Consists in first extracting with absolute alcohol under a reflux, evaporating and defatting the extract. This is boiled with water and a few drops of lead acetate solution and then filtered, using diatomite. The glycoside is next decomposed by heating with hydrochloric acid, and finally the strophanthidin is thoroughly extracted with chloroform and weighed. This multiplied by 2.187 gives the weight of strophanthin represented. A similar process is used for the tincture.—W. Kroseberg, *Pharm. J.*, i/1914, 590.

Strophanthinum (B.P.). Adjusted by admixture with lactose to possess an activity equal to 40% of that of anhydrous ouabain. Loss on drying in a vacuum desiccator over sulphuric acid, not more than 3%. Ash, not more than 1%. Samples are compared with a standard preparation of strophanthin the potency of which has been accurately determined in relation to anhydrous ouabain. The method used for assay is one of those described under *Digitalis Pulverata*. *Strophanthinum, U.S.P. XII*, possesses a potency per mg. equivalent to 0.5 mg. of *U.S.P.* ouabain reference standard. It is assayed by the cat method described for *Tinctura Digitalis, U.S.P. XII*.

Wokes (*Quart. J. Pharm.*, 1928, 513) examined a series of commercial samples, testing them in comparison with ouabain, and found them to vary from 25 to 60% of the potency of ouabain.

Distinction between Strophanthin and Ouabain. Strophanthin is soluble in water 1 in 40 to 43 at 15°. The aqueous solution of this glycoside, unlike that of ouabain, gives a persistent froth on agitation.

The following distinguishing colour test has been proposed: 5 ml. of conc. hydrochloric acid, a few crystals of resorcinol and a trace of the glycoside are warmed to 60° or 70°. Ouabain gives no coloration, while strophanthin gives a rose colour.—*J. chem. Soc. Abstr.*, ii/1921, 601.

Hispidus strophanthin, like *kombé* strophanthin, is a mixture of two glycosides.—*Brit. chem. Abstr.*, Dec., 1928, 1376.

Strophanthin-G (Ouabain). This is the official strophanthin of the German Pharmacopœia and is included in the *U.S.P. XII*. It is referred to in the *B.P.* as a standard for biological assays. It is not an official preparation in the Pharmacopœia because supplies for therapeutic use are believed to be insufficient. It is a crystalline substance of constant composition except that it may contain different amounts of water of crystallisation. The international standard ouabain contains 20% of water of crystallisation. On crystallisation at the ordinary temperature $C_{21}H_{44}O_{11} \cdot 9H_2O$ is obtained, whereas if the temperature is 30° or 60° the crystals contain four and three molecules of water respectively. Ouabainum, *U.S.P. XII*, has the formula, $C_{21}H_{44}O_{11} \cdot 8H_2O = 728.77$. M.p., 186° to 189°; α_D determined on a 1% w/v aqueous solution, -31° to -32.5°; loss on drying at 130°, 18 to 22%. Complies with tests for absence of alkaloids.

Spinal rather than anæsthetised cats should be used for the assay of cardiac glycosides. In the assay of strophanthin by the cat method, the grouping of the figures is improved if a non-volatile anæsthetic be used instead of ether, and still further improved by using spinal animals. The interval between the preparation of the cat and the infusion should be about 4 hours.—A. D. MacDonald, *Quart. J. Pharm.*, 1934, 182.

SULPHANILAMIDUM

Sulphanilamidum (B.P. Add. IV and VI). $C_6H_8O_2N_2S = 172.1$. Contains 99.0 to 100.5% of the pure compound, calculated with reference to the dried substance. Loss in *vacuo* at 100°, not

more than 1%. Dissolves completely in 10 parts of dilute hydrochloric acid, and in 5 parts of sodium hydroxide solution (10%). No ammonium salts should be detected on boiling with sodium hydroxide solution. Assayed by formation of the di-bromo substitution product by interaction with excess N/10 bromine solution in acid mixture during 15 minutes, interaction of the excess bromine with potassium iodide and back titration with N/10 sodium thiosulphate; 1 ml. of N/10 bromine is equivalent to 0.0043 g. $C_6H_8O_2N_2S$. Sulfanilamidum, *U.S.P. XII*, loses not more than 0.5% at 100°, and then contains not less than 99% of $C_6H_8O_2N_2S$. M.p., 164.5° to 166.5°. Assayed by dissolving in hydrochloric acid and water, cooling to 15°, adding crushed ice and slowly titrating with M/10 sodium nitrite whilst stirring vigorously, until the titration mixture after standing for 1 minute gives an immediate blue colour when streaked on a smear of starch iodide paste. Ash, not more than 0.1%.

Alternative Assay Methods.—The following methods are suggested:—

(a) As the di-bromo substitution product. 30 to 80 mg. is dissolved in acid in a flask, the stopper of which is surrounded by a cup, the solution is made alkaline with sodium hydroxide, diluted to 50 ml., treated with 0.5 g. of potassium bromide and then with N/10 potassium bromate run in from a burette until there is a 10 to 30% excess. 10 ml. of hydrochloric acid is added and the flask closed and placed in the dark for 5 minutes. It is then cooled, a solution of 3 g. of potassium iodide in 10 ml. of water is run in from the cup, the mixture is diluted to about 300 ml. and titrated with N/10 thiosulphate. 1 ml. of potassium bromate solution is equivalent to 4.304 mg. of sulphanilamide. Errors varied from +0.3 to +0.8%.

(b) *Direct titration.* Sulphanilamide in solution in 5-10% sulphuric acid may also be determined by direct titration with potassium bromate solution after adding 1 g. of potassium bromide. The end-point is indicated by the appearance of a yellow colour. The results are somewhat low.

(c) *Hydrolysis of the Sulphonamide Group.* 20 to 200 mg. is boiled for 1 hour with 5 ml. of 70% sulphuric acid beneath a reflux condenser with ground glass connections. The condenser is rinsed into the flask the contents of which are then diluted to 50 to 60 ml. A few drops of methyl red solution and a little pumice are added and the liquid distilled into a receiver containing a measured volume of N/50 to N/10 hydrochloric acid, according to the weight of sample being tested. When the air in the apparatus has been replaced by water vapour, 10% solution of sodium hydroxide (ammonia-free) is added from a tap funnel until there is an excess of 0.2 to 1 ml. over that required for neutralisation. When two-thirds of the liquid has been distilled, the condenser is rinsed into the receiver, the contents of the latter are boiled with pumice, cooled and titrated with N/50 or N/10 sodium hydroxide. 1 ml. of N/50 acid is equivalent to 8.4428 mg. of sulphanilamide. The error varies from 0 to +1.2%.—E. Schulek and I. Boldizsar, *Z. anal. Chem.*, 1937, 108, 396.

The sulphur may be determined by the following process:—Introduce a weighed quantity of the sample (about 0.15 g.) into a flask containing 2 g. of potassium permanganate, 0.5 g. of potassium hydroxide and 30 ml. of water, and boil the mixture under a reflux condenser for three hours. The contents of the flask are then carefully acidified with hydrochloric acid and boiled until a clear solution is obtained, then diluted with water and the sulphate determined in the usual way by precipitation with barium chloride.—N. L. Allport, *Quart. J. Pharm.*, 1936, 560.

Two rapid and accurate methods are described for the assay of sulphanilamide. The first is a determination of the crystallising point and the second, the diazotisation method performed potentiometrically.—R. W. Towne and R. M. Hitchens, *J. Amer. pharm. Ass.*, 1939, 585.

About 0.5 g. of sulphanilamide is treated with 20 ml. of concentrated hydrochloric acid in a beaker, and the mixture brought to the boil. While boiling gently, 5 ml. of hydrogen peroxide (30%) is dropped in during a period of ten

minutes. The solution is boiled for two minutes and filtered, and the sulphate in the filtrate determined by precipitation with barium chloride.—G. v. Mikó, *Pharm. Zentralh.*, 1939, 80, 198.

Microscopical Identification. Description of technique and appearance of crystals.—C. Van Zyp, per *Analyst*, 1938, 5, 511.

A table of the aqueous solubilities of sulphanilamide over a temperature range of 23° to 50° is given.—R. H. Keirle and J. M. Sayward, *J. Amer. chem. Soc.*, 1942, 2465.

Tabellæ Sulfanilamidi (U.S.P. XII). Contain 95 to 105% of the labelled amount of $C_6H_5N_2O_2S$, including all tolerances. A weighed quantity of powdered tablets is assayed by titration with M/10 sodium nitrite as described for sulphanilamide.

Determination in Tablets. Weigh 20 tablets, transfer them to a clean mortar, and powder. Weigh 0.4 g. of the powder and transfer it to a 25 ml. glass-stoppered graduated cylinder. Add 10 ml. of an ice-cold, filtered, saturated solution of sulphanilamide. Shake well. Filter through a Jena glass filter. Wash any residue in the cylinder and the material on the filter with a few drops of ice-cold distilled water to displace any sulphanilamide solution. Completely extract the residue in the cylinder and the material on the filter with 80 to 100 ml. of hot alcohol. Wash the funnel tip and any exposed edges with a few ml. of hot alcohol. Collect the alcohol and washings in a tared 250-ml. beaker. Carefully evaporate the alcohol on a steam-bath. Dry in the oven at 80° to constant weight.—W. E. Housinger and R. E. Schoetgow, *J. Amer. pharm. Ass., Sci. Edn.*, 1940, 133.

A tentative method for the determination of sulphanilamide is described in *Methods of Analysis (A.O.A.C., 1940, 614)*. The sample containing about 0.5 g. of sulphanilamide is placed on a filter and the soluble portion washed into a flask with about 25 ml. of acetone. The acetone is removed by warming to 70° and 10 to 12 ml. of 75% v/v sulphuric acid added. The solution is slowly refluxed for thirty minutes, the condenser washed down with water, the solution diluted to about 100 ml. with water, and an excess of 50% alkali added. The solution is distilled, the ammonia in the distillate collected in an excess of N/10 sulphuric acid and back titrated with N/10 sodium hydroxide, using methyl red as indicator. 1 ml. of N/10 sulphuric acid is equivalent to 0.01722 g. of sulphanilamide.

Sulphacetamidum (B.P.C. Supp. III). $C_8H_{10}O_3N_2S = 214.1$. Loses not more than 0.5% on drying at 100°, and then contains 99.5 to 100.5% of $C_8H_{10}O_3N_2S$. M.p., 181° to 183°. Ash, not more than 0.1%. Assayed by titration at a temperature below 5° of the acidified solution with M/10 sodium nitrite, using freshly prepared starch potassium iodide paper as external indicator. 1 ml. M/10 sodium nitrite is equivalent to 0.02141 g. of $C_8H_{10}O_3N_2S$.

Sulphapyridina (B.P.C. Supp. III). $C_{11}H_{11}O_2N_3S = 249.2$. Determined by titration with M/10 sodium nitrite at a low temperature, contains 99 to 100.6% of the pure substance calculated on the dried substance. Loss at 100°, not more than 0.5%. M.p., 191° to 193°. Sulfapyridinum, *U.S.P. XII*, when dried at 100°, contains not less than 99% $C_{11}H_{11}N_3O_2S$. Ash, not more than 0.1%; 1 g. forms a clear, colourless solution in approximately N/20 sodium hydroxide. Sulfapyridinum Sodicum Sterile, *U.S.P. XII*, loses not more than 7% at 105° and then contains not less than 99% $C_{11}H_{11}N_3O_2SNa$. Complies with the sterility tests for solids, *U.S.P. XII*.

Tabellæ Sulfapyridini (U.S.P. XII). Contain 95 to 105% of the labelled amount of $C_{11}H_{11}N_3O_2S$, including all tolerances. Assayed by titrating a weighed quantity of powdered tablets with M/10 sodium nitrite as described for sulphanilamide.

Test for Sensitivity of Pneumococci to Sulphapyridine. Pneumococci differ profoundly in their sensitivity to the sulphonamides; resistant strains occur in nature; sensitive strains may become resistant when they are exposed to the drug; resistant strains are probably permanently resistant. A rapid qualitative test is as follows: each of four mice is inoculated intraperitoneally with 0.2 ml. of 10% sulphapyridine suspended in 20% acacia. Fresh sputum (or empyema pus) obtained from the patient is made grossly homogeneous and liquid by mixing with a minimum necessary amount of sterile saline solution. Within the first two hours after treatment the four treated and in addition three untreated mice are alternately inoculated with the sputum mixture, as large a volume as possible (up to 1 ml.) being used for each mouse. At various intervals thereafter peritoneal exudate is removed from each mouse and examined rapidly to determine the number and morphology of the pneumococci. The test is read when growth of the pneumococci in the untreated mice has become unquestionable, and at no earlier time. If, at this time, the treated mice show no increase in the number of pneumococci, or if there has been scanty growth with a preponderance of atypical forms, the strain is called sensitive; if smears of treated and control mice are virtually indistinguishable, the strain is called resistant. The test requires an average of 18 hours for completion and has a sharp end-point.—F. J. Moore *et al.*, *J. Amer. med. Ass.*, ii/1941, 437.

Sulphathiazolum (B.P.C. Supp. III). $C_9H_9O_2N_3S_2 = 255.2$. Assayed similarly to sulphapyridine it contains 99.4 to 100.6% of $C_9H_9O_2N_3S_2$. M.p., 202° to 203° . Loss at 100° , not more than 0.5%. Ash, not more than 0.05%. Sulfathiazolum, *U.S.P. XII*, contains not less than 99% $C_9H_9N_3O_2S_2$ after drying at 100° . M.p., 200° to 204° ; ash, not more than 0.1%. Complies with tests for acidity and solution in sodium hydroxide solution. Assayed by titration with M/10 sodium nitrite.

The colorimetric method of estimation of sulphapyridine (Marshall, *Science*, 1938, 88, 85) is applicable to sulphathiazole. The following test distinguishes between sulphathiazole and sulphapyridine. To a solution of the sodium salt add a solution of cupric sulphate, when sulphathiazole produces a purple and sulphapyridine an apple-green precipitate.—W. A. Lott and F. H. Bergheim, *J. Amer. chem. Soc.*, 1939, 3593.

The method depends upon the production of a stable, easily soluble, violet component by coupling of sulphathiazole with 1-sulphomethylaminonaphthalein-8-sulphonic acid and the determination is carried out colorimetrically. Details are given of the application of the method to blood, urine and milk determinations.—J. Druey and G. Oesterheld, *Analyst*, 1942, 331.

Tabellæ Sulfathiazoli (U.S.P. XII). Contain 95 to 105% of the labelled amount of $C_9H_9N_3O_2S_2$, including all tolerances. Assayed by the process described for sulphanilamide using M/10 sodium nitrite.

Sulphadiazine, sulphapyrimidine or 2-sulphanil-aminopyrimidine, melts at 255° to 256° with decomposition. It is soluble in water at 37° , 12.3 mg. per cent. The acetyl derivative is approximately identical, with slightly higher m.p. and solubility.—*Amer. Prof. Pharm.*, 1941, 365.

Sulphaguanidine, sulphanilylguanidine, is a fine, white, tasteless powder, which crystallises in water as the monohydrate. It melts at 142.5° to 143.5° . The anhydrous drug softens at 168° to 178° and melts at 189° to 190° . Soluble in boiling water 10%, boiling alcohol (95%) 5%, in dilute mineral acids; insoluble in dilute alkalis. A saturated solution in water at 25° has pH 6.9. Chemically it has two possible formulas, thought to be resonance isomers:— α -sulphanilylguanidine is $NH_2 \cdot C_6H_4 \cdot SO_2 \cdot NH \cdot C : NH \cdot NH_2$, and β -sulphanilylguanidine is $NH_2 \cdot C_6H_4 \cdot SO_2 \cdot NH : C(NH_2)_2$.—*Amer. Prof. Pharm.*, 1941, 361.

Sulphathiophene, 2-sulphanilamido-thiophene, has been prepared as white needle-like crystals, m.p. 156.5° to 157.5° , soluble in hot water, methyl alcohol, ethyl alcohol, 1% aqueous sodium hydroxide, 1% aqueous hydrochloric acid and slightly in ether.—R. W. Bast and C. F. Starnes, *J. Amer. chem. Soc.*, 1941, 1885.

SULPHONAL

Sulphonal (B.P.). $(\text{CH}_3)_2\text{C}(\text{SO}_2\text{C}_2\text{H}_5)_2 = 228.2$. M.p., 125° to 127° ; ash, not more than 0.05%. A test for readily oxidisable substances is included. The *N.F. VII* substance melts between 124° and 126° and loses not more than 1% when dried over sulphuric acid. The *Fr. Cx.* limits the ash to 0.1% and includes tests for heavy metals, barium and chloride.

Methylsulphonal (B.P.). $(\text{CH}_3)(\text{C}_2\text{H}_5)\text{C}(\text{SO}_2\cdot\text{C}_2\text{H}_5)_2 = 242.3$. M.p., 76° to 78° ; ash, not more than 0.05%. Complies with a test for readily oxidisable substances. Sulfonethylmethanum, *N.F. VII*, melts between 74° and 76° .

A tentative method for the determination of sulphonal and methyl sulphonal is described in *Methods of Analysis (A.O.A.C., 1940, 581)*.

SULPHUR

Sulphur Præcipitatum (B.P.). S = 32.06. Residue on ignition, not more than 0.5%. Consists, when examined microscopically, of grouped amorphous globules without crystalline particles. The *U.S.P. XII* substance, dried over sulphuric acid, contains not less than 99.5% of S. Assayed by oxidation of a solution in potassium hydroxide with hydrogen peroxide, and precipitation as barium sulphate, subtracting the weight obtained in a blank determination on the reagents used. Residue on ignition, not more than 0.3%.

Sulphur Sublimatum (B.P.). Residue on ignition, not more than 0.25%. Consists, when examined microscopically, chiefly of almost opaque, rounded, amorphous particles or aggregates, occasionally associated with semi-crystalline masses. The *U.S.P. XII* substance, dried to constant weight over sulphuric acid and assayed by precipitation as barium sulphate, contains not less than 99.5% of S. Residue on ignition, not more than 0.5%.

Detection of Free Sulphur. Free sulphur can be detected by using benzylimido-di-(*p*-methoxyphenyl)-methane, $(\text{CH}_3\cdot\text{O}\cdot\text{C}_6\text{H}_4)_2\text{C}:\text{N}\cdot\text{CH}_2\cdot\text{C}_6\text{H}_4$. The reagent is used as the solid. The substance to be tested is well ground with the reagent and a portion of the mixture is placed in a micro test-tube and heated for 5 minutes in an oil-bath at 210° . The tube is then removed from the bath and 2 to 3 drops of benzene are added. If sulphur was originally present the benzene is coloured blue owing to the formation of *pp'*-dianisylthioketone. On adding a small crystal of mercuric chloride, the blue colour slowly fades and the crystal acquires a red or orange colour.

Assay of Preparations. The sample to be assayed, containing 0.1 g. of S, is weighed accurately into a 175 ml. conical flask and 50 ml. of a solution containing 40 g. of potassium cyanide A.R., 90 ml. of triethanolamine and water to 1000 ml., is added together with 1 g. of soft paraffin. A little pumice is added to prevent superheating, and the mixture is vigorously boiled for $\frac{1}{2}$ hour under a reflux condenser. The liquid is cooled, treated with 10 ml. formaldehyde solution and acidified with dilute nitric acid, and 50 ml. of N/10 silver nitrate is added. The mixture is decolorised if necessary with charcoal, filtered, the residue washed, and the filtrate titrated with N/10 ammonium thiocyanate, using ferric alum as indicator. A blank is carried through on 10 ml. of the reagent. Each ml. of N/10 AgNO_3 is equivalent to 0.003206 g. of S.—N. L. Allport, *Quart. J. Pharm.*, 1933, 431.

Sulphur in flowers of sulphur, sulphur ointment, confection of sulphur, sulphur tablets and compound liquorice powder may be determined by boiling the material (equivalent to about 0.1 g. of sulphur) in a conical flask attached to a reflux condenser with 2 g. of crystalline sodium sulphite and 30 to 40 ml. of water until all the sulphur has dissolved. The addition of 1 g. of soft paraffin assists solution. Cool, pour off from the paraffin and wash the residue with water, add 10 ml. of solution of formaldehyde and 10 ml. of 20% acetic acid to remove the excess of sulphite, and titrate the thiosulphate formed in the solution with N/10 iodine, using starch as indicator; 1 ml. of N/10 iodine is equivalent to 3.206 mg. of sulphur.—H. R. Fleck and A. M. Ward, *Quart. J. Pharm.*, 1934, 179.

Sulphur Ointment. About 0.5 g. is weighed in a 50 ml. flask of resistant glass, 2 ml. of 20% w/v sodium hydroxide solution is added and the flask heated on a small hole of the water-bath. From time to time the flask is gently rotated so as to wash down any particles of sulphur from the sides which if necessary may be finally rinsed with a fine jet of hot water. After $\frac{1}{2}$ to 1 hour the sulphur dissolves completely, both oily and aqueous layers being clear. 5 ml. of 20 vol. hydrogen peroxide is added, and the flask again heated on the water-bath for 15 minutes, a small funnel being placed in the neck of the flask to avoid loss by effervescence. About 20 ml. of hot water is added, and the liquid cautiously acidified with dilute (1 : 3) hydrochloric acid, care being taken to avoid a large excess (4 to 5 ml. is required). The liquid is then filtered through a wet filter-paper into a beaker and the flask washed thoroughly with several quantities of hot water, the washings being passed through the same filter. Sulphate in the filtrate is determined in the usual way by precipitation with barium chloride. A blank test is made with the reagents and the results deducted. The method can be applied to confection of sulphur (using about 0.1 g.) and sulphur lozenge (about 0.15 g., previously powdered). It is also applicable to compound liquorice powder, but the sample after being weighed in the flask is preferably moistened with 1 ml. of water which is allowed to soak in before adding the alkali.—A. N. Leather, *Analyst*, 1936, 335.

The following assay is recommended by the Sub-Committee on the Assay of Galenicals of the Committee on Pharmaceutical Chemistry:—Boil about 1 g., accurately weighed, with a solution of 2 g. of crystalline sodium sulphite in 40 ml. of water in a 250-ml. conical flask under a reflux condenser until the sulphur is completely dissolved. Cool, decant the aqueous solution, wash the residue of fat with hot water; cool, decant and repeat the washing. Add the washings to the solution; add 10 ml. of solution of formaldehyde and 6 ml. of acetic acid; dilute to 150 ml. and titrate with N/10 iodine, using mucilage of starch as indicator. Each ml. of N/10 iodine is equivalent to 0.003206 g. of S. Limits of 9.5 to 10.5% of S are suggested.—(*British Pharmacopæia Commission Report*, No. 11, May 1939.)

Mustard Gas, dichlorodiethyl sulphide, $S(CH_2CH_2Cl)_2$, was first used at Armentières by the Germans in 1917 and is generally considered to be the most powerful nuisance gas on account of its prolonged delayed action and owing to its odour frequently passing unnoticed. It is a heavy oily liquid, colourless when pure, but when impure the colour varies from a pale yellow to a tarry black. The sp. gr. is 1.28 at 15°, so that it sinks in water, but on wet surfaces it spreads, forming an iridescent film, from which vapour is given off. It has a m.p. of 14.4° and readily supercools, and the freezing-point may be depressed, if necessary, by mixing with organic solvents. The b.p. is 217°, with decomposition. It is only slightly soluble in water, but is almost miscible with most organic solvents other than mineral oils, in which it is not so soluble. It is one of the most stable of war gases and is only slowly hydrolysed by cold water, though it is readily attacked by most oxidising agents, such as bleaching powder, potassium permanganate and chloramine-T, and these are employed for its destruction.

Detection. Mustard gas reacts with many alkaloidal reagents, yielding a turbidity of colloidal gold with 0.1% aqueous gold chloride solution, and precipitates with potassium tri-iodide, potassium mercury iodide and potassium bismuth iodide. It forms a di- β -naphthyl ether with a 1% solution of β -naphthol in N/10 50/50 aqueous alcoholic alkali forming a white turbidity, which becomes more obvious on warming. With sodium iodoplatinate solution iodine is liberated and this can be detected by the addition of starch solution, but the official method, which has now been adopted and which is known as the S.D. test, is dependent on the formation of acid which turns Congo red paper blue.

Sulphuretted Hydrogen in Industry.

This gas is formed when organic matter containing sulphur decomposes. It causes deaths in sewers, occurs in mines, and is a danger in certain chemical industries, especially gas, coke and tar works and petroleum refineries. It is colourless and has an odour of bad eggs by which it is readily detected, but the sense of smell is rapidly paralysed and lost after 2 to 15 minutes exposure at a concentration of 1 in 10,000. If concentrated it may resemble carbon monoxide in its rapidity of action, the subject appearing to drop almost instantaneously and it is considered one of the most toxic of gases. In concentrations of 1 in 1000 or higher it is nearly as toxic as hydrogen cyanide. 1 in 2000 is very dangerous if inhaled for 15 to 30 minutes and causes severe irritation of the eyes and respiratory tract with risk of pneumonia or serious injury to lungs which may prove fatal. 1 in 5000 is dangerous if inhaled for 1 hour, the eyes being affected after 6 to 8 minutes. 1 in 10,000 causes the eyes and respiratory tract to be affected after exposure for 1 hour.

Treatment. Prompt removal from the contaminated area, followed by artificial respiration, if necessary with administration of oxygen to which 5 to 7% of carbon dioxide has been added. The patient must be kept warm.

Detection and Determination. Hydrogen sulphide is most readily detected by means of lead acetate paper. Filter paper is impregnated with a 10% solution of lead acetate in 5% acetic acid and dried in a hydrogen sulphide-free atmosphere. The outer portions of the paper are discarded and a known volume of the suspected air is drawn through the filter paper by means of a pump or an aspirator, the colour varying from a yellowish-brown to a dark brown or black according to the quantity of gas present. The colour developed may be compared with those given in official standard charts. (*Toxic Gases in Industry*, No. 1, D.S.I.R.)

Sulphuris Chloridum (B.P.C.). S_2Cl_2 = 135.0. Sp. gr., about 1.70. B.pt., about 138°. Solidifying-point, about -80°.

Sulphuris Iodidum (B.P.C.). Contains not less than 70% of I. Assayed by titration of a triturate with potassium iodide solution, with N/10 sodium thiosulphate.

Calx Sulphurata (B.P.C.). Contains not less than 50% of CaS. Assayed by the *B.P.C.* process:—1 g. mixed with 10 ml. of water, 25 ml. of copper sulphate solution, and 10 ml. of dilute hydrochloric acid is warmed on a water-bath for 15 minutes, cooled and diluted to 100 ml. 25 ml. of the filtered mixture (the first 25 ml. is rejected) with 1 g. of potassium iodide is titrated with N/10 sodium thiosulphate, using starch mucilage as indicator; a blank experiment is performed.

Calcium sulfuraturn solutum, *P. Helv. V* (Soluté de Vlemingkx), contains 6% *w/v* of polysulphide sulphur. *Assay:* Boil for 2 minutes in a 100 ml. wide-mouthed flask 60 ml. of water with 1 g. of boric acid and a few pieces of porous pot. Add rapidly and cautiously 0.2 g. of potassium cyanide and then 1 ml. of the sulphur solution, and boil for 10 minutes. After cooling, transfer to a 100 ml. graduated flask, and add sufficient water to make 100 ml. Take 50 ml. of this solution, acidify with 1 or 2 ml. of concentrated hydrochloric acid and add bromine water until a yellow colour persists in the solution. Remove the excess of bromine by adding phenol solution and allow to stand for 10 minutes; add 1 g. of potassium iodide, and after 15 minutes titrate the liberated iodine with N/10 sodium thiosulphate, using starch as indicator towards the end of the titration. Each ml. of N/10 thiosulphate is equivalent to 0.001603 g. of polysulphide sulphur.

Lime-Sulphur Solution. The specification of the Association of British Insecticide Manufacturers (Bulletin No. 82; Ministry of Agriculture and Fisheries) for lime-sulphur solution requires it to be clear and free from sludge and of sp. gr. about 1.3, with not less than 18.5% *w/w* of polysulphide sulphur equivalent to about 24% *w/v*.

Potassa Sulphurata (B.P.). Contains 42 to 45% of total sulphur assayed by precipitation as sulphate from a solution in sodium hydroxide solution oxidised with bromine solution. Potassa Sulphurata, *U.S.P. XII*, contains not less than 12.8% of S in combination as sulphide; assayed by addition of a copper sulphate solution to a solution of the substance, filtering and adding hydrogen sulphide solution to the filtrate, acidified with acetic acid; no black precipitate should be produced.

Kalium sulfuraturn crudum, *P. Helv. V*, should contain not less than 18% of sulphur polysulphides by the given process. Dissolve 2 g. in water and adjust to 50 ml. Also mix separately 60 ml. of water, 1 g. of boric acid and a few fragments of pumice, boil and add first 0.15 g. of potassium cyanide and at once 5 ml. of the sulphurated potash solution and boil for 10 minutes. Cool and adjust to 100 ml. Acidify 50 ml. with 1 to 2 ml. of hydrochloric acid, add bromine water till just coloured, removing the excess with phenol solution. After 10 minutes add 1 g. of potassium iodide, stand for 15 minutes and titrate with N/10 sodium thiosulphate. 1 ml. of N/10 $\text{Na}_2\text{S}_2\text{O}_3 = 0.001603$ g. of polysulphides.

Mesulphenum (B.P.C. Supp. III). $\text{C}_{14}\text{H}_{18}\text{S}_2 = 244.2$. Contains 23.0 to 26.2% of S, determined by digestion with fuming

nitric acid in a sealed tube at 300° for six hours and weighing as barium sulphate. Sp. gr., 1.20 to 1.22. A test for chloride and inorganic sulphide is included.

THYMOL

Thymol (B.P.). $C_{10}H_{14}O=150.1$. M.p., 48° to 51°. Residue on evaporation from an open dish on a water-bath, not more than 0.05%. The solution in alcohol is neutral to litmus and optically inactive. Oily drops do not separate on standing from a 20% w/v solution in 10% w/v sodium hydroxide solution, which is clear and colourless or pale red. The *Fr. Cx.* 1937 determines the purity of thymol by precipitation as thymol iodide in alkaline solution. Thymolum, *P. Helv. V*, by treatment with N/10 bromide-bromate solution, hydrochloric acid and carbon tetrachloride during 30 minutes, addition of potassium iodide and titration with N/10 sodium thiosulphate, contains 99% of $C_{10}H_{14}O$.

An official method for the determination of thymol is described in *Methods of Analysis (A.O.A.C., 1940, 600)*. A solution of thymol in dilute alkali is warmed with hydrochloric acid and standard bromine solution, methyl orange indicator is added and the titration with bromine solution completed. Details are also given for the determination of thymol in antiseptics.

Thymolis Iodidum (B.P.C.). $C_{20}H_{24}O_2I_2=550.0$. By the B.P. method described under Iodophthaleinum, the substance dried over sulphuric acid contains not less than 40% of I. Loss over sulphuric acid, not more than 5%. Sulphated ash, not more than 3%. Thymolis Iodidum, *U.S.P. XII*, contains, after drying over sulphuric acid, not less than 43% of iodine. Assayed by igniting with potassium carbonate, oxidising the solution with potassium permanganate, decolorising with alcohol, filtering, adding potassium iodide to a portion, acidifying and titrating with sodium thiosulphate. Dithymol-di-iodé, *Fr. Cx.* 1937, assayed by the given process, contains not less than 99% of the pure substance. Dissolve, with gentle heat, 40 ml. of sulphuric acid, 1.5 g. of silver nitrate and 5 g. of potassium dichromate; after cooling again, add 0.25 g. of the thymol iodide and warm (not above 150°); after cooling, add 150 ml. of water and pass in sulphur dioxide, filter off the silver iodide, dry and weigh.

Oleum Thymi (B.P.C.). By the B.P. method for eugenol in Oleum Caryophylli, it contains not less than 40% w/v of phenols. Sp. gr., 0.905 to 0.960; $n_{D_{20}^{\circ}}$, 1.483 to 1.510. Soluble in 2 volumes of alcohol (80%). Oleum Thymi, *N.F. VII*, contains not less than 20% v/v of phenols. Sp. gr. at 25°, 0.894 to 0.930. Assayed by measurement of the volume of oil unabsorbed by potassium hydroxide solution. Oleum Thymi, *P. Helv. V*, contains 20 to 42% of phenols (thymol and carvacrol); assayed by interaction during 30 minutes of the portion soluble in sodium hydroxide solution, with N/10 bromide-bromate solution, carbon tetrachloride and hydrochloric acid; followed by titration of the iodine, liberated from

potassium iodide, with N/10 sodium thiosulphate. The *Fr. Cx.* 1937 requires Essence de Thym to contain not less than 25% of phenols, calculated as thymol, by an assay process similar to that of the *P. Helv. V* but without the addition of carbon tetrachloride.

Thyme. The Food and Drug Administration of the U.S. Dept. of Agriculture defined thyme as the dried leaves and flowering tops of *Thymus vulgaris* L., containing not more than 14% of total ash, nor more than 4% of ash insoluble in hydrochloric acid.—*S.R.A., F.D., No. 2, Rev. 5, Nov. 1936.*

Oleum Ajowan (B.P.C.). By the *B.P.* method for eugenol in Oleum Caryophylli, the unabsorbed oil measures not more than 6 ml., equivalent to not less than 40% *v/v* of thymol. Sp. gr., 0.910 to 0.930; α_D , 0° to +2°; n_{D20} , 1.485 to 1.510.

Sage. The Food and Drug Administration of the U.S. Dept. of Agriculture defined sage as the dried leaf of *Salvia officinalis* L., containing not more than 12% of stems (excluding petioles) and other foreign matter.—*S.R.A., F.D., No. 2, Rev. 5, Nov. 1936.*

THYROIDEUM

Thyroideum (B.P. Add. I). Contains 0.09 to 0.11% of iodine in combination as thyroxine. Assayed by precipitation during 18 to 24 hours of an N/1 sodium hydroxide extract with sufficient N/1 sulphuric acid to render the mixture slightly acid to congo red, followed by ignition with sodium carbonate of the precipitate previously dried with sodium carbonate at 110°; after extraction of the residue with water, neutralisation with sulphuric acid, oxidation with bromine, etc., as in the assay process for Thyroxinsodium, the liberated iodine is titrated with N/200 sodium thiosulphate. Thyroideum, *U.S.P. XII*, contains 0.17 to 0.23% of iodine in thyroid combination, and is free from iodine in inorganic or any other form of combination than that peculiar to thyroid gland. Thyroide, *Fr. Cx.* 1937, dried at 105°, contains not less than 0.2% of organic iodine. Moisten 1.1 g. in a nickel crucible with 1 ml. alcohol, add 5 ml. of 20% pure potash solution and after 3 to 4 hours, stirring occasionally, evaporate and ignite, at first gently and then more strongly till completely incinerated. After cooling, dissolve in water, and re-ignite. After dissolving in boiling water, filter and wash till 200 ml. are obtained. To the colourless solution add 10 ml. of 2% potassium permanganate, boil for 10 minutes, decolorise the excess with alcohol, cool, dilute to 220 ml. and filter. To 200 ml. of filtrate add 10 ml. of acetic acid and 1 g. ammonium chloride and boil for 10 minutes to destroy nitrites. To the cooled liquid, add 10 ml. of acetic acid and 1 g. potassium iodide and after 5 minutes titrate with N/100 sodium thiosulphate (recently prepared from N/10). Water-soluble ash, not more than 4%; insoluble ash, not more than 3.5%.

Glandulæ Thyreoidæ siccatae, *P.G. VI*, is standardised to contain not less than 0.18% of iodine. Thyreoides siccata, *P. Helv. V*, is prepared by drying the chopped glands below 50° and adjusting the defatted powder, if necessary, with lactose to contain

from 0.08 to 0.1% of thyroxine. The product should contain not more than 2% of fat, 8.5% of moisture, and yield not more than 5% of ash.

To avoid confusion, it should be borne in mind that thyroid *B.P.*, though standardised to 0.1% of iodine in combination as thyroxine, actually contains more than 0.2% of total iodine. Of the three iodines present in thyroid, only one, acid-insoluble thyroxine iodine, is recognised in the *B.P.* product, and it is on thyroxine iodine content solely that the *B.P.* standardisation is determined.—Armour & Co., *Brit. med. J.*, ii/1932, 780.

The use of a cold water extract of desiccated thyroid for the determination of the inorganic iodine as advocated in the *B.P.* '32 is inapplicable in the case of preparations which have been desiccated at low temperatures, since a considerable proportion of the thyroglobulin will not be denatured and will be extracted by the water.—H. G. Rees and A. H. Salway, *Quart. J. Pharm.*, 1932, 627.

A preparation obtained by low-temperature desiccation is most readily brought into line with the *B.P.* assay process by leaving in contact with alcohol for 17 hours and drying at 55° to 60°.—C. R. Harington and S. S. Randall, *ibid.*, 629.

Total iodine and acid-insoluble iodine determinations in thyroid gland are described. The standardisation of thyroid on a basis of acid-insoluble iodine content requires only one determination of iodine. It is unnecessary to fix a standard for total iodine content, and determinations of "inorganic" iodine no longer serve any useful purpose.—G. Middleton, *Analyst*, 1932, 603.

Inorganic iodides in dry thyroid may be determined by shaking 0.2 to 3 g. for 2½ hours in a stoppered tube with 20 ml. of cold methyl alcohol, filtering and washing with methyl alcohol; the extract is evaporated and the iodine in the residue determined.—W. Lawson, *Biochem. J.*, 1933, 112.

Recent experiments indicate that most of the iodine is bound to protein, but can be extracted from the protein by butyl alcohol. This extract, in turn, can be separated into "thyroxin-like" and "diiodotyrosine-like" fractions. Salter has suggested the following classifications of iodine fractions in the plasma: (1) "I" iodine, non-precipitable and presumably inorganic, and (2) "P" iodine, the maximum amount precipitated with the protein. The "P" fraction may then be separated into a "T" (thyroxin-like fraction) and a "D" (diiodotyrosine-like fraction).—T. Lerman, *J. Amer. med. Ass.*, ii/1941, 349.

The faults found with Hunter's method, as modified in the *U.S.P. X*, are loss of iodine on acidifying the solution of carbonates with phosphoric acid, and the introduction of oxychlorine compounds, including chloric acid, by the use of sodium hypochlorite solution. These compounds also liberate iodine from potassium iodide, and are not eliminated by boiling. With due care during neutralisation the first-mentioned source of error can be minimised, and where a fair proportion of iodine is present, error due to oxychlorine compounds is negligible. Where the iodine content is considerably less than 0.2%, a modification of Kendall's method is best. One gramme of the substance is gently heated with powdered caustic soda in a nickel crucible over an Argand burner. The crucible is heated more strongly until all the organic matter is oxidised, but no nitrate is added. The melt is extracted with water and filtered. Using bromophenol blue as indicator, the mixture is neutralised with phosphoric acid (sp. gr., 1.75). Excess bromine water is added, and an excess of 2 ml. of phosphoric acid. The liquid is boiled to half its volume to expel bromine, the remaining traces being eliminated by adding salicylic acid after cooling. The iodine is liberated from the iodate by the addition of potassium iodide solution and the iodine titrated with N/200 thiosulphate.—Wilfred Smith, *Quart. J. Pharm.*, 1928, 372.

The following method is stated to be preferable to fusion with alkali, as in *P. Ned. V*, and to alkaline oxidation methods. 200 mg. of sample is dissolved in 5 ml. of warm 4N sodium hydroxide and the solution shaken with 200 mg. of talc. 50 ml. of 4% potassium permanganate and 25 ml. of 4N sulphuric acid are added, and the solution heated in a 300 ml. Erlenmeyer flask, on which is a mark indicating 75 ml., until it begins to froth, after which it is boiled gently for 5 minutes. The mixture is cooled, about 3 g. of sodium bisulphite added to destroy the excess of permanganate, and the liquid boiled with 3 ml. of N/10 silver nitrate to remove SO_4 . The solution is boiled with 15 ml. of 50% nitric acid, filtered when cold, and the residue washed until free from acid. The precipitate

is washed back into the original flask, the liquid diluted to 75 ml., and 5 ml. of dilute sulphuric acid and 10 ml. of fresh bromine water added to convert silver iodide to the bromide and the iodine to iodate. The excess of bromine is boiled off and the liquid titrated with N/100 sodium thiosulphate. 1 ml. of N/100 thiosulphate solution is equivalent to 0.212 mg. of iodine.—J. C. de Jong, *Pharm. Weekbl.*, 1937, 1429.

The variation in the total iodine and thyroxine iodine contents of fresh gland and dried defatted glands is so great that there can be no satisfactory correlation between thyroid B.P. '32 and preparations expressed in terms of fresh gland or unstandardised dried defatted gland.

Biological Methods of Assay. There are three biological methods for estimating the activity of thyroid. An early method was that introduced by Reid Hunt, known as the acetonitrile test, which depends on the rise in resistance to acetonitrile produced in mice by the administration of thyroid. A dose of acetonitrile injected into each of a group of mice will kill a certain percentage. If the mice have previously received thyroid the same dose will kill a smaller percentage. Two samples of thyroid can thus be compared by observing what reduction in the percentage each causes. The relative potency of the two samples is, of course, not directly proportional to the respective reductions in percentage mortality, but can be determined from previous experiments in which the effect of different doses of the same sample of thyroid has been determined.

A second method of observing the physiological action of thyroid is to determine its effect on oxygen consumption, or on carbon dioxide production. The oxygen consumption of guinea-pigs, rabbits or rats can be estimated by placing the animal in a metal chamber immersed in water at constant temperature. The chamber contains a soda-lime tower to absorb carbon dioxide, and oxygen is admitted as required through a bubble trap. The passage of each bubble of oxygen is recorded on a smoked paper by a mechanical device, and the number of bubbles in a given period of time is a measure of the oxygen consumption. The volume of a bubble is previously determined when calibrating the instrument. The administration of thyroid raises the oxygen consumption, and different samples of thyroid can be compared by finding what doses produce the same rise in oxygen consumption.

A third method of estimating the potency of thyroid is by means of its effect in causing loss of body weight in guinea-pigs. Two groups of six guinea-pigs may be taken and their weights observed from day to day. One sample of thyroid is administered to one group, daily for six days, while a second sample, to be compared with the first, is administered to the second group. The mean loss of weight in each group can then be determined. The more potent sample produces the greater loss in weight; the relative potency of two samples is most rapidly found by finding a dose of one which produces an effect lying between the effects produced by two doses of the other.

Tabellæ Thyroidei (U.S.P. XII). Contain iodine equivalent to 0.17 to 0.23% of the labelled amount of thyroid, including all tolerances. Assayed by mixing a weighed quantity of powdered tablets with alcohol, sodium hydroxide solution and anhydrous sodium carbonate, drying and heating at 525° to 550° for 20 minutes, extracting the residue with water and filtering the extract. Bromine solution is added to the warm filtrate, the mixture allowed to stand 5 minutes, and then boiled to remove excess bromine. After cooling, phosphoric acid and potassium iodide solution are added and the liberated iodine titrated with N/10 sodium thiosulphate. A blank determination is carried out on the materials used.

Thyroxinsodium (B.P. Add. I). $C_{15}H_{10}O_4NI_4Na = 798.8$. Contains from 61 to 65% of I. The B.P. Add. I assays Thyroxinsodium by direct ignition with sodium carbonate, extraction of the residue with water, neutralisation with sulphuric acid (50% v/v) and oxidation by boiling for 10 minutes with 1 ml. of the sulphuric acid, 0.2 ml. bromine and adding a small piece of marble; after cooling, 0.2 ml. of phenol in glacial acetic acid (25% w/v) is added, followed by potassium iodide and titration with N/20 sodium thiosulphate, using starch mucilage as indicator; 1 ml. of N/20 sodium thiosulphate = 0.001058 g. of I. Thyroxinum,

U.S.P. XII, contains not less than 64% of iodine; it is assayed by igniting the substance, dried over sulphuric acid for 24 hours, with potassium carbonate, treating the water extractive with potassium permanganate, discharging the colour with alcohol, filtering, and titrating a portion of the filtrate, after adding potassium iodide and acid, with N/200 sodium thiosulphate.

Thyroxine (*Fr. Cx.* 1937). $C_{15}H_{11}NO_4I_4=776.8$. Assayed similarly to Thyroïde, *Fr. Cx.*, contains not less than 60% of iodine. Limit of diiodotyrosine, as matter insoluble in aqueous mixture adjusted with hydrochloric acid to pH 3.5, not more than 2%.

Parathyroid Extract.

Biological Method of Assay. The potency of parathyroid extract has been determined by injecting it subcutaneously into dogs to observe whether a rise in the amount of serum calcium occurs. This method suffers from the disadvantage that a given dose produces a different effect in different dogs, and the maximum rise occurs at a variable time. A more accurate method has been proposed by Dyer, who found that the injection of the extract into rats caused a rise in the amount of urinary calcium. Dyer (*Quart. J. Pharm.*, 1933, 426) uses ten rats which are placed in separate cages to allow collection of the urine. The output of calcium is determined, the urine of five rats being pooled, and the urine of the other five also being pooled. These observations are made for two or three days. The one group of rats is then injected with the extract which is used as standard, and the second group with the preparation which is being tested. The injections are given daily for two or three days. If the potency of the doses given is exactly equal, the rise in output of calcium in the two groups is the same.

If the potency of the doses is not exactly equal, the dose with the greater potency produces the greater rise in urinary calcium. But it is not possible to calculate the relative potency of doses which produce unequal rises in calcium output. When unequal rises occur, the experiment must be repeated until a dose of the unknown preparation is found which produces the same rise as a dose of the standard.

Injectio Parathyroidei (*U.S.P. XII*) replaces *Liquor Parathyroidei U.S.P. XI*. 1 ml. possesses a potency of not less than 100 *U.S.P.* parathyroid units; each unit representing 1/100 of the amount required to raise the calcium content of 100 ml. of normal dog blood serum by 1 mg. within 16 to 18 hours after administration.

TRAGACANTHA

Tragacantha (*B.P.*). Foreign organic matter, not more than 2%. The powder does not acquire a pink colour in ruthenium red solution. Ash limit, 4%. *Tragacantha, U.S.P. XII*, complies with a test for karaya gum.

The following tests for identity of tragacanth are recommended by the Subcommittee on Crude Drugs of the Committee on Pharmacy and Pharmacognosy:—To 4 ml. of a 0.5% *w/v* solution in water, add 0.5 ml. of hydrochloric acid and heat for thirty minutes in a water-bath. Divide the liquid into two parts. To one part add 1.5 ml. of solution of sodium hydroxide and 3 ml. of solution of potassium-cupric tartrate and warm in a water-bath; a red precipitate is produced. To the remainder of the liquid add solution of barium chloride; no precipitate is produced (distinction from agar). When powdered, it does not acquire a pink colour in solution of ruthenium red (distinction from sterculia gum and from agar). On the addition of N/10 iodine, it acquires an olive-green colour (distinction from acacia and from agar).—(*British Pharmacopæia Commission Report*, No. 13, September 1939.)

The following test provides a means of comparing specimens of tragacanth, but it is necessary that tests should be carried out at the same time and under exactly the same conditions if comparative results are to be obtained:—The tragacanth should be in the form of powder, or, if in flake form, should be

reduced to a powder which passes a No. 30 sieve and is retained by a No. 60 sieve. Prepare a mucilage of 1.25% strength as described in the *B.P.* for *Mucilago Tragacanthæ*; heat for 1 hour on a boiling water-bath, with occasional stirring, pour it into a 50 ml. Nessler cylinder and allow it to stand overnight. At the surface of this mucilage release a steel ball, $\frac{5}{32}$ in. in diameter, and take the time of fall from a point 1.5 in. to a point 4 in. below the upper surface; the time required is from 50 to 150 seconds for average specimens of tragacanth when freshly powdered. Occasional specimens give much higher results.

The comparison between samples of gum tragacanth should be made by comparison of the viscosities of 0.4% aqueous mucilages in poises at 20.0°. If a gum gives a mucilage with a viscosity above 1.3 poises it may be regarded as of good quality.—*L. A. Haddock, Quart. J. Pharm.*, 1934, 505.

Its most important character is its power to form a mucilage, yet no test, such as the falling ball method, is included in the *B.P.* for distinguishing different qualities.—*Pharm. J.*, ii/1932, 208.

The quantity of volatile (acetic) acidity developed in the acid hydrolysis of gum tragacanth affords a valuable index of the purity of this commodity when compared with results obtained from so-called "Indian gum." A tentative method for the determination of volatile acidity is described.—*Methods of Analysis (A.O.A.C. 1940, 601)*.

UREA

Urea (*B.P.C.*). Melts between 130° and 132°. Ash, not more than 0.1%. M.p. of the *U.S.P. XII* substance, 131° to 133°. Alcohol-insoluble matter, not more than 0.04%.

Urethanum (*B.P. Add. IV*). $\text{NH}_2\text{COOC}_2\text{H}_5 = 89.06$. M.p. of the substance dried over sulphuric acid, 48° to 50°. Ash, not more than 0.05%. *Æthylis Carbamas*, *U.S.P. XII*, melts between 48° and 50°. Loss on drying over sulphuric acid, not more than 2%; ash, not more than 0.1%. 1 g. in 5 ml. of alcohol forms a practically complete and colourless solution. Tests for nitrate and urea are included.

Carbromalum (*B.P.*). $\text{C}_7\text{H}_{13}\text{O}_2\text{N}_2\text{Br} = 237.0$. M.p., 116° to 118°. Complies with limit tests for carbonisable substances, chlorides and sulphates. Ash, not more than 0.05%.

Suraminum (*B.P. Add. IV*). $\text{C}_{51}\text{H}_{34}\text{O}_{23}\text{N}_6\text{S}_6\text{Na}_6 = 1428.6$. pH of a 1% solution in recently boiled and cooled water, 6.2 to 6.8, using bromothymol blue indicator. Contains in 5 g. free amine equivalent to not more than 0.4 ml. of N/10 sodium nitrite. Complies with tests for absence of undue toxicity, as indicated by intravenous injection of mice, and for therapeutic potency as indicated by intravenous injection of mice infected with a strain of *Trypanosoma equiperdum*.

VALERIANA

Valeriana (*B.P. Add. IV*). Foreign organic matter, not more than 5%. Ash, not more than 12%. The *B.P. Add. IV* allows the use of Indian valerian when valerian is prescribed or demanded. The *N.F. VII* requires a foreign matter limit of 4% and an acid-insoluble ash limit of 8%.

A water-soluble base possessing physiological activity is present in appreciable quantity in dried valerian root. The base is soluble in cold and hot water, and in alcohol, sparingly soluble in ethyl acetate, and insoluble in acetone, chloroform and ether. It is precipitated from a solution in amyl alcohol by ether. When distilled in high vacuum it decomposes at 200° . It has not yet been crystallised. The vapours evolved on heating the base with zinc dust did not give the characteristic red colour on pine wood of a pyrrole derivative. Physiologically it caused a fall in blood pressure in an urethane-anesthetised cat followed by a secondary effect on the heart; it also had an inhibiting effect on peristalsis. These effects require confirmation when the basis obtained is pure. Details are given for the separation of the base.—*Pharm. J.*, i/1939, 299.

Valeriana Indica (B.P. Add. IV). Contains not more than 2% of other foreign organic matter. Ash, not more than 12%.

Ammonii Valeras Acidus (N.F. VII). By distillation of the ammonia liberated with sodium hydroxide it yields the equivalent of from 33 to 38% of ammonium valerate, $C_4H_9 \cdot COONH_4$; and by titration with standard alkali to phenolphthalein, the acidity corresponds to 62 to 67% of free valeric acid, $C_4H_9 \cdot COOH$.

Ferri Valerianas (B.P.C.). Residue on ignition, not less than 24%.

Sodii Valerianas (B.P.C. Supp. IV). $C_5H_9O_2Na = 124.1$. Contains not less than 85% of the pure substance. Assayed by titration of a solution with N/2 sulphuric acid to bromophenol blue removing most of the acid produced with ether.

Zinci Valerianas (B.P.C.). $C_{10}H_{18}O_4Zn, 2H_2O = 303.6$. On treatment with nitric acid and gentle ignition, yields 25 to 28% of ZnO .

ZINCUM

Zinci Carbonas (B.P.C.). Residue on ignition, not less than 68%.

Determination of Zinc. A slightly acid solution containing zinc, free from metals other than the alkalis or alkaline earths, and from ammonium acetate, is made slightly alkaline with sodium hydroxide, then acidified with acetic acid and diluted so that above 0.1 g. of zinc is present in each 150 to 200 ml. Excess of sodium anthranilate solution is then added slowly with shaking, which is continued for 3 to 5 minutes. After standing for 20 minutes the zinc anthranilate is filtered off, washed with sodium anthranilate solution, then with alcohol and dried at 105° to 110° . 1 g. of precipitate corresponds to 0.2411 g. of zinc oxide or 0.1937 g. of Zn. The reagent is prepared by dissolving 3 g. of anthranilic acid in dilute sodium hydroxide solution in amount just insufficient to neutralise and diluting to 100 ml. The zinc solution is best prepared by precipitating the zinc as sulphide, igniting the precipitate to oxide and dissolving the latter in the minimum quantity of hydrochloric acid.—E. A. Ostroumow, per *Analyst*, 1937, 690.

Zinc can be determined by means of 8-hydroxyquinoline, (*syn.* Oxine), $C_8H_7N \cdot OH$. To the test solution, which should be slightly acid and should contain about 0.05 g. of Zn in 50 ml., is added 3 g. to 5 g. of sodium acetate. After warming to 60° to 70° , the zinc is precipitated by the addition of excess of the alcoholic 8-hydroxyquinoline solution. The precipitate is collected, washed and dried. If dried at 100° , the composition is $Zn(C_8H_6ON)_2, 1\frac{1}{2}H_2O$ and the Zn content is 17.18%; at 120° to 130° the crystals become anhydrous, and then contain 18.50% of Zn. The precipitate may also be determined volumetrically as described for magnesium. Each millilitre of N/5 bromine is equivalent to 0.001635 g. of Zn.

The oxine complex can be weighed as the dihydrate after drying at 98°. After drying at 160° the weight of the precipitate corresponds to the crystalline anhydrous form. The slightly high results at 110° are therefore associated with residual dihydrate, and at 140° presumably with the retention of water by the anhydrous form in the amorphous condition. The practical point emerging is that instructions need to be more precise than, say, "dry at 100°"; the hydrated form may possibly be retained by drying in a steam oven, but the more certain procedure is to dry the anhydrous form at 160°.—R. C. Chirnside, C. F. Pritchard and H. P. Rooksby, *Analyst*, 1941, 399.

Zinc in Food. Determination of zinc in foods using diphenylthiocarbazone. Results are given for a variety of food materials.—N. D. Sylvester and E. B. Hughes, *Analyst*, 1936, 734.

Zinci Oxidum (B.P.). $\text{ZnO} = 81.38$. Loses not more than 1% on ignition, and then contains not less than 99% of ZnO . Assayed by dissolving the substance and ammonium chloride in excess N/1 sulphuric acid and back titrating with N/1 sodium hydroxide to methyl orange. The *U.S.P. XII* omits the ammonium chloride in the titration to methyl red indicator, and requires a purity of 99% in the freshly ignited substance.

Emplastrum Zinci Oxidi (B.P.C.). Weight per sq. yd. not less than 8 oz., and of the base cloth $3\frac{1}{2}$ oz., and the difference between these weights not less than $3\frac{1}{2}$ oz. The cotton cloth complies with the standard for the cotton cloth of *Emplastrum Adhesivum*. Tensile strength of the warp, not less than 45 lbs. per inch width.

Unguentum Zinci Oxidi (U.S.P. XII) is assayed by ignition and weighing the greyish-white residue of oxides. 18.5 to 21.5% of ZnO should be indicated.

Calamina (B.P.C. Supp. IV). Residue on ignition, 68 to 90%. Matter insoluble in hydrochloric acid, not more than 1%. The filtrate from 2 g. shaken with 20 ml. of water and 5 ml. of glacial acetic acid remains clear on addition of 5 drops of potassium chromate solution (limit of lead) and the filtrate from 0.5 g. shaken with 5 ml. of water and 1.5 ml. glacial acetic acid, made ammoniacal and precipitated with hydrogen sulphide, on acidification with acetic acid and addition of ammonium oxalate solution produces no turbidity (limit of calcium and barium salts). *Calamina Præparata, N.F. VII*, loses on ignition not more than 1% and then, by titration of the filtrate from solution in excess standard sulphuric acid with standard sodium hydroxide to methyl orange, contains not less than 98% of ZnO .

The following tests for purity are recommended by the Sub-Committee on Inorganic Chemicals of the Committee on General Chemistry:—Dissolve 1 g. in 20 ml. of warm dilute hydrochloric acid, filter, and wash with water; the residue after being dried at 100° weighs not more than 0.01 g. (limit of matter insoluble in hydrochloric acid). Shake 1 g. with 10 ml. of water and filter; the filtrate is colourless (absence of water-soluble dyes). Shake 1 g. with 10 ml. of alcohol (90%) and filter; the filtrate is colourless (absence of alcohol-soluble dyes). Dissolve 2 g. in a mixture of 20 ml. of water and 5 ml. of glacial acetic acid, filter, and add 5 drops of solution of potassium chromate; the solution remains clear (limit of lead). Dissolve 1 g. in a mixture of 10 ml. of water and 2.5 ml. of glacial acetic acid, filter, make alkaline by the addition of solution of ammonia, and add 2 ml. of solution of ammonium oxalate; no turbidity is produced (limit of calcium and soluble barium salts). 0.5 g. dissolved in water by the addition of 3 ml. of nitric acid, complies with the limit tests for chlorides. 0.2 g. dissolved in water by the addition of 2 ml. of hydrochloric acid, complies

with the limit test for sulphates. Leaves, on ignition, not less than 68%, and not more than 72%, of residue.—(*British Pharmacopœia Commission Report*, No. 14, September 1939.)

Nebcalamina Præparata (N.F. VII). Contains, after ignition, not less than 92% ZnO. In the assay, iron is removed by precipitation with excess ammonium hydroxide, and the filtrate, after addition of slight excess HCl, is titrated against N/10 potassium ferrocyanide, using ferrous ammonium sulphate as indicator.

Zinci Oleostearas (B.P.C.). By the B.P. method for Zinci Stearas, it contains 12 to 14% of zinc, calculated as ZnO.

Zinc (Peroxyde de) (Fr. C.x. 1937) by titration with potassium permanganate contains not less than 35% ZnO₂.

Zinci Stearas (B.P.). Contains zinc equivalent to from 13 to 15.5% of ZnO. Assayed by boiling in excess of N/10 sulphuric acid for 10 minutes, filtering and titrating the filtrate and washings with N/10 sodium hydroxide to methyl orange. The U.S.P. XII substance, assayed similarly, is of the same strength.

Titanic Chloride, TiCl₄, is kept in sealed tubes. It reacts violently with water and forms a white precipitate. On adding ammonia complete precipitation of the hydroxide occurs.

Titanous Chloride, TiCl₃, is supplied in 15% solution which is used as a reducing agent and employed in volumetric analysis for the determination of iron and of organic nitro-compounds. N/10 solution is prepared by diluting approximately 1 volume with 1 volume of concentrated hydrochloric acid and 8 volumes of water and standardising immediately before use with N/10 ferric ammonium sulphate. It should be freshly prepared, or preserved in an atmosphere of hydrogen or carbon dioxide.

Titanic Oxide, TiO₂. A white powder, extremely insoluble in ordinary solvents. It does not dissolve in boiling nitric acid or aqua regia. Used as an ingredient of toilet powders and as a white pigment in place of "White Lead." RUTILE is the ore titanium dioxide used in leather dyeing.

Titanous Sulphate, Ti₂(SO₄)₃, usually supplied in 15% solution, is employed as a reducing agent.

Detection of Tin. Small amounts of tin may be detected by means of diazine green (*syn.* Janus green). The reagent is used as a 0.01% aqueous solution and the test is especially useful for the detection of tin in the presence of antimony in the sulphides obtained in systematic qualitative analysis. The sulphides are dissolved in strong hydrochloric acid and reduced with metallic iron. One drop of the solution is added to 1 ml. of the reagent. In the presence of tin there is a colour change from the blue to violet or red.

Tin may be detected by using α -dinitrophenylaminesulphoxide, NH(C₆H₄.NO₂)₂SO. The reagent is used as a 0.2% solution in N/10 sodium hydroxide. The tin is precipitated from the test solution by means of hydrogen sulphide and the liquid filtered. The precipitate and filter are boiled with concentrated hydrochloric acid, the solution filtered and to the filtrate is added a piece of zinc foil. While the zinc is dissolving, a stream of carbon dioxide is passed through the liquid. When the metal has dissolved, 2 ml. of reagent is added and the mixture heated for 2 minutes, carbon dioxide still being passed through it. The liquid is diluted with water, 1 to 2 drops of ferric chloride solution added and the liquid filtered. If tin is present, the filtrate is coloured violet. For the detection of tin in, for example, canned foods the organic matter must first be destroyed by boiling with sulphuric acid and potassium bisulphate.

Determination of Tin in Foods. A distillation method for quantitative separation and estimation of tin in foodstuffs is described:—Incinerate 5 to 20 g. material, according to the amount of tin suspected, in a silica dish. Transfer the charred mass to a distillation flask and transfer any residue in the dish by digestion with sulphuric acid A.R. Make up volume in flask to 30 ml. with sulphuric acid A.R. Connect the flask to a water-cooled condenser attached to three small wash bottles, each containing 5 ml. of water. Heat the distillation flask in an oil bath to 220° and drop hydrobromic acid (sp. gr., 1.46–1.49) on to the contents of the distillation flask, at the rate of 1–2 drops per second. Meanwhile, maintain a steady current of dry CO₂ through the absorption flasks. Continue the distillation for 30 to 45 minutes. Rinse the condenser and dilute the distillates to 50 to 100 ml., according to amount of tin suspected.

Colorimetric Determination.—Take an aliquot portion of distillate containing 20–25 μ g. of tin and add just sufficient phenol (25% in glacial acetic acid) to render it free from bromine. Determine the acidity by titration with N sodium hydroxide using methyl orange indicator. For the test, take a further aliquot portion, and add phenol reagent and the calculated amount of 30% sodium hydroxide solution. Add 4 drops of thioglycollic acid (0.04% aqueous solution) to reduce stannic tin, 3 to 4 drops of a fairly stiff agar agar mucilage and 1 ml. of 0.1% solution of toluene-3:4-dithiol in 1% *w/v* solution of sodium hydroxide. Make up the total volume to 10 ml. Heat the solution in boiling water for 1 minute and compare the resulting colour with those given by standard tin solutions, similarly treated.—H. N. Law, *Analyst*, 1942, 283.

Detection of Molybdenum and Tungsten. To 10 ml. of the solution to be examined, containing between 1 and 0.02 mg. of molybdenum or tungsten, add sodium hydroxide or hydrochloric acid until the mixture is neutral to litmus, and then 10 drops of hydrochloric acid and 3 drops of thioglycollic acid. Molybdenum gives a bright yellow solution, tungsten a colourless solution. Mix this solution with 1 ml. of dimercaptobenzene (dithiol) reagent, and boil for 3 to 4 minutes. Molybdenum gives a dark green precipitate and tungsten a light greenish-blue precipitate. Finally, cool the mixture and add an excess of ammonia (0.880). A bright blue colour indicates molybdenum, a colourless solution tungsten. Iron interferes with the test and if present should first be precipitated by sodium hydroxide and filtered off.—J. H. Hamence, *Analyst*, 1940, 152.

ZINGIBER

Zingiber (B.P.). Alcohol (90%)-soluble extractive, not less than 4.5%. Water-soluble extractive, not less than 10%. Ash limit, 6%. Water-soluble ash, not less than 1.7%. Zingiber, *U.S.P. XII*, yields not less than 4.5% of ether-soluble extractive and not less than 12% of cold-water extractive.

The Food and Drug Administration of the U.S. Dept. of Agriculture defined ginger as the washed and dried, or decorticated and dried, rhizome of *Zingiber officinale* Roscoe. Required to contain not less than 42% of starch, not more than 8% of crude fibre, not more than 1% of lime, not less than 12% of cold-water extract, not more than 7% of total ash, not more than 2% of ash insoluble in hydrochloric acid, nor less than 2% of ash insoluble in cold water. *Jamaica Ginger*: Ginger grown in Jamaica, containing not less than 15% of cold-water extract and otherwise as ginger. *Limed Ginger*, bleached ginger: whole ginger coated with calcium carbonate, containing not more than 4% of calcium carbonate nor more than 10% of total ash, and otherwise as ginger.—*S.R.A., F.D., No. 2, Rev. 5, Nov. 1936.*

Proportion of Extractive. The darker outer layers of ginger yield much higher proportions of extractive to alcohol and to ether than are yielded by the inner portions. Four samples of (scraped) Jamaica ginger gave the following ranges of percentage yields for the outer and inner portions respectively:—Alcohol, 4.4–5.0, and 2.4–2.8; ether, 5.5–6.7 and 3.0–3.5. With African ginger (5 samples) the figures were:—Alcohol, 6.5–9.1 and 3.4–4.0; ether, 9.2–13.0, and 4.2–4.9. With Cochin ginger (5 samples) the figures were:—Alcohol, 6.0–8.2 and 2.5–3.2; ether, 8.4–11.2 and 2.8–4.0. Only 3 out of 13 samples of exhausted ginger showed gelatinised starch granules and since low values for the extractive may be due to the removal of the outer layers, it is not possible to detect the presence of small admixtures of exhausted ginger with certainty. It may be assumed that a sample with an alcoholic extract of less than 3.0% is adulterated. One with an alcoholic extract of 3.0–4.5% may be genuine but the fibre should be less than about 3.0% and the aqueous extract not less than about 12%.—G. D. Elsdon and C. Mayne, *Analyst*, 1937, 836.

PROPRIETARY MEDICINES

An early attempt to control the advertising and sale of proprietary medicines for which extravagant therapeutic claims were made was the publication by the British Medical Association of *Secret Remedies: What they cost and what they contain* (1909), followed by *More Secret Remedies* (1912). In 1912 the House of Commons appointed a Select Committee to inquire into the conditions prevailing in the United Kingdom regarding the sale and advertising of proprietary medicines, medical preparations and appliances. The Report of the Committee was issued in 1914 and was reprinted as a supplement to the *Lancet*, January 10, 1925 ("Sale of Patent Medicines.") It was found that this country differed from the Dominions and most foreign countries in having no Government Department or official charged with the duty of controlling the advertising and sale of proprietary remedies, and that the existing law offered no check to gross abuse of the public. It was recommended that the administration of the law governing the advertising and sale of patent, secret and proprietary medicines be part of the functions of the Ministry of Health. The Committee considered that the exhibition of formulæ was not a "proper, practical or effective measure." The reasons given for taking this view were, firstly, that such a procedure would inflict grave hardship, sometimes amounting to ruin, upon proprietors of secret remedies and losses of their investments upon shareholders. Secondly, that the disclosure of composition would be meaningless to most purchasers. Thirdly, that it was the duty of the State to protect subjects against injury and fraud. Experience during recent years of the practice of "de-stamping" (*vide infra*) has shown that the fear that disclosure of formulæ might involve the proprietors of secret remedies in ruin was completely groundless. The Committee further suggested that pure drugs, vended entire under fancy names, should no longer be exempt from duty, that the distinction between the name of an ailment and the name of an organ, the seat of that ailment, should be abandoned, and that the exemption should not apply to medicines generating carbonic acid gas. It was also recommended that reference to the patent medicine stamp in advertising matter should be prohibited and that no name of a proprietor or firm should be printed on the stamp. The report also dealt with the law relating to proprietary medicines in the British Dominions, Germany, Austria, Hungary, France and Italy.

Proprietary Medicine Bills

In July, 1920, a Bill based on the recommendations of the Select Committee was introduced into the House of Lords. Among the proposals were the prohibition of the sale or advertising of remedies purporting to treat or cure certain diseases and the registration of proprietary medicines and their owners. The

proprietary medicine register was to be in two parts, one of which was to be used exclusively for recording the composition of the medicines. Authority was to be given to the Minister to require the disclosure on the label of any poisonous or dangerous ingredient, but apart from this no disclosure was to be required. Powers were to be given to remove a medicine from the register if it was considered injurious to health.

A Proprietary Medicine was defined as any medicine held out by advertisement, label, or otherwise in writing, as efficacious for the prevention, cure or relief of any malady, ailment, infirmity or disorder affecting human beings and

- (a) which is sold under a trade name or trade mark to the use of which any person has or claims or purports to have an exclusive right; or
- (b) of which any person has or claims or purports to have the exclusive right of manufacture or for the making of which any person has or claims or purports to have any secret.

For an analysis of the provisions of the Bill as amended in the Committee of the House of Lords, see *Chem. & Drugg.*, ii/1920, 1472; see also *ibid.*, ii/1920, 1503 and i/1921, 49.

After reaching the Report stage in the House of Lords the Bill was not proceeded with on account of the dissolution of Parliament. A further Bill, with essentially the same provisions, was drawn up by the Health Advisory Committee of the Labour Party and introduced in the House of Commons in May 1931, but was afterwards withdrawn.

Throughout the discussions on the Bill exception was taken to the disclosure of formulæ of proprietary medicines, but the position in this respect was altered completely by "de-stamping" (see below).

At the request of the Standing Committee on Scientific Research of the Economic Advisory Council, the Council of the Royal College of Surgeons gave their views on patent medicine legislation, the following being abstracted from a statement appearing in the 1934 Annual Report of the College.

"In the opinion of the Council the problem can only be adequately dealt with on the lines of the Select Committee of 1914 by bringing the sale of proprietary medicines and appliances under the control of the Ministry of Health. Such control should ensure: (1) that the article is not injurious; (2) that the description of its therapeutic action is not fraudulent; (3) that the sale of the article and the method of its advertisement are not against the public interest; (4) that no medicine or appliance is advertised as a cure for any of the following diseases and conditions:— blindness, Bright's disease, cancer, consumption, diabetes, epilepsy, fits, locomotor ataxy, lupus, paralysis."—per *Brit. med. J.*, ii/1934, 822.

In Canada a proprietary or patent medicine is defined by the Proprietary or Patent Medicines Act as "any artificial remedy or prescription manufactured for the internal or external use of man, the main composition or definition of which is not to be found in

the *British Pharmacopæia* . . . or other recognised and approved pharmacopœias or formularies . . . or upon which is not printed in a conspicuous manner the true formula or list of medicinal ingredients contained in it."—*Pharm. J.*, ii/1934, 590.

Medicine and Surgical Appliances (Advertisement) Bill

In 1936 a Bill with the above title was introduced into the House of Commons as a private member's Bill. The following were the principal provisions of this measure:—

- (1) Prohibition of holding out a medicine or appliance, whether directly or by implication (a) as a cure of Bright's disease, cancer, consumption, diabetes, epilepsy, fits, locomotor ataxy, lupus, or paralysis; (b) as a cure of amenorrhœa, hernia, blindness, or any structural or organic ailment of the auditory system; (c) for procuring miscarriage; (d) for the cure of habits associated with sexual excess, or for the promotion of sexual virility in men or of sexual desire in women.
- (2) Prohibition of publication of invitations to diagnosis by correspondence or to treatment of the above ailments by correspondence.
- (3) Provision was made to permit of advertisements of medicines for the above ailments being sent to medical practitioners and others.

When the Bill came up for second reading in March 1936, opponents suggested that it would unduly restrict the activities of the unorthodox practitioner, and that it was designed to safeguard the business of qualified doctors and chemists far more than to safeguard the public. Eventually, it was found that a quorum could not be obtained, and the House was "counted out." The British Advertising Association, which includes the proprietors of many of the most extensively advertised remedies has, however, adopted the criteria contained in this Bill and all advertisements of members of the Association must now conform to them.

Proprietary Medicines and Revenue

The immense increase in the sale of patent medicines since the Select Committee of 1912 was appointed is indicated by the fact that in 1928-9 the *revenue alone* from Medicine Stamp Duty reached a maximum of £1,333,512, whereas Dr. Cox, before the Select Committee (1912), made the statement (*Chem. & Drugg.*, i/1912, 923) that £2,500,000 had been *spent by the public* on patent medicines since 1908. The Report (1937) of the Select Committee on Medicine Stamp Duties suggested that, at a conservative estimate, the annual (retail) turnover in proprietary medicines was not less than £20,000,000. The number of patent medicine licence holders had increased from 29,000 in 1903 to 190,000 in 1934. Since 1929 the yield from the duty had rapidly decreased owing to the practice of de-stamping, and amounted only to £747,930 in 1935-6.

"De-stamping"

The Schedule to the Medicine Stamp Act, 1812, exempted from duty, when sold by chemists or doctors, any preparation containing ingredients of which the "different denominations, properties, qualities, virtues and efficacies . . . are known, admitted, and approved of in the preservation, cure, or relief of any disorder . . . in anywise affecting the human body," provided that no claims were made by manufacturer or vendor to the possession of a special right to prepare the medicine or of special skill in compounding the ingredients. A further condition of this exemption was that the medicine must not be and must never have been held out by the owner, compounder or first vendor as a cure for a disease. Once a medicine was liable to duty it was always liable, but this rule was easily evaded by making some slight alteration in the formula.

In order to take advantage of these exemptions, the majority of proprietary medicine manufacturers supplied, for sale by chemists, unstamped articles the formulæ of which were printed on the label; such preparations were vended as, say, "—Brand Cough Mixture." The brand name was registered as a trade-mark, which conferred proprietary rights on the *name*, although the label bore some statement such as "Proprietary rights are not claimed in the formula or method of preparation of this article except in the registered trade-mark." The majority of extensively advertised proprietary medicines were sold unstamped under brand names, the formulæ being disclosed.

Prior to 1930 the Commissioners of Customs and Excise had always regarded the exemption of known, admitted and approved remedies as applying to preparations of which the formula was published in a recognised work of reference (such as the *B.P.*, *B.P.C.*, or the *Extra Pharmacopœia*), provided the label bore an indication of the source of the formula. In 1930, they issued a notice requiring a "definite and complete statement of the actual ingredients (i.e., the full formula with proportions), and a statement that no proprietary rights are claimed in the medicine" to be printed on labels, cartons, etc. In 1932 an attempt to enforce this ruling in the Courts was unsuccessful (*Attorney-General v. Lewis and Burrows*), and exemption could be successfully claimed if the label indicated where the formula might be found in a well-known book of reference and proprietary rights were disclaimed.

Select Committee on Medicine Stamp Duties

The decline in revenue due to de-stamping led in 1936 to the appointment of a Select Committee "to consider the duties of excise chargeable under the Medicine Stamp Acts, and to make recommendations for the alteration of those duties, if thought fit, with a view to reforming the law relating thereto." The Committee, which reported in 1937, found that the existing law was quite inappropriate to modern requirements and that wholesale evasion of duty was practised. They considered that the trade in

medicinal articles was suitable for taxation, that the same product should not be sold by different vendors stamped and unstamped, and that a far larger range of preparations should be made liable to duty. The Committee proposed that duty should be chargeable on practically every type of preparation, whether a medicine, toilet requisite, cosmetic, article of confectionery, or medicated wine, which was recommended, held out or advertised in any way whatsoever for the prevention, cure or relief of any human ailment, etc., or for the protection or maintenance of bodily health. They proposed that exemption be granted to medicines recommended and sold to doctors and dentists, to medicines supplied to doctors and dentists for professional use and to all preparations supplied to doctors, dentists or pharmacists for dispensing purposes. They further urged that consideration should be given to the propriety of taxing foods and certain appliances (such as deaf aids), beverages and other preparations advertised as possessing properties beneficial for health. It was suggested that if difficulty was experienced in distinguishing between cosmetics which claim to be remedial and those which do not, consideration should be given to the taxation of all cosmetics. The rate of duty proposed was $\frac{1}{2}$ d. for articles retailing at $\frac{3}{4}$ d., exclusive of duty, rising to 1d. for those retailing from 3d. to 6d., and 1d. for every 6d. thereafter.

The sweeping character of these proposals evoked vigorous opposition from the interests affected. Among the objections raised to the suggestions were the necessity of stamping medicines dispensed in accordance with doctors' prescriptions and the fact that simple remedies such as Epsom salts would be dutiable if, at the time of sale, any recommendation was made as to their therapeutic effect.

Pharmacy and Medicines Act, 1941.

In April 1939 the Chancellor of the Exchequer, in opening the Budget, announced that the troublesome question of the Medicine Stamp Duties was to be settled finally by their abolition as from September 2nd, 1939, a date chosen to enable the existing licences to expire as they normally did on September 1st and also to give time to allow sufficient opportunity for the disposal of existing stocks. The cost to the Exchequer was estimated as £770,000 in a full year. Considerable opposition was aroused on a number of grounds. The proposal destroyed the long established pharmacists' privilege in respect of "known, admitted, and approved remedies" as no alternative was proposed and, if the duty was removed without some form of control, there would be no inducement to disclose the composition of any preparation. About two hundred Members of Parliament signified their intention to support an amendment to the Finance Bill with the object of postponing the repeal of the duties for five years. In the subsequent debate the Chancellor yielded to the wishes of the House and agreed to the postponement of the repeal for twelve months. At the same time he invited the House to assist him in

devising a workable measure to replace the obsolete enactments. The parliamentary discussion is reported in the *Pharmaceutical Journal* for July 1st, 1939. The Chancellor of the Exchequer, in the following August, announced that he and the Minister of Health jointly would appoint a Committee to consider the control and taxation side of the question. For the year ending March 31st, 1939, the total net receipts were £725,640, a figure £7000 lower than 1938 and the lowest since 1934-5. The number of licences issued had increased by about 3500 to a total of 167,371. It was, however, not until the introduction of the Budget in April 1941 that the Chancellor of the Exchequer was able to announce that he proposed to repeal the medicine stamp duties as from September 2nd, 1941. Sir Kingsley Wood said:

"The Committee will also remember that when the Finance Bill of July was being debated the question was raised of the application of the Purchase Tax to medicines subject to medicine stamp duty. It was pointed out that this involved a tax upon a tax, and comments were made upon the medicine stamp duties themselves which, as my predecessor told the House in 1939, had become administratively unworkable, and which it was highly desirable to repeal on that ground. I undertook before the introduction of the next Finance Bill to see whether the parties could be got together to hammer out some reasonable solution of the position.

"I am glad to be able to inform the Committee that the various interests concerned with the duties, assisted by my hon. and gallant Friend the Member for the Lonsdale Division of Lancashire (Sir Ian Fraser), have come together and have reached an agreement. I have consulted my right hon. Friend the Minister of Health on the matter, and as a result I am in a position to announce that the medicine stamp duties will be repealed and that my right hon. Friend, in accordance with the understanding which has been reached, will as soon as possible introduce legislation, the object of which will be to maintain a fair balance between the interests of the pharmacists and of other vendors. The repeal will come into operation from September 2nd so as to allow reasonable time for disposal of stamped stocks. We shall part therefore with the Act of 1802 and other Acts on this matter which in process of time have become a museum piece of administrative complexity. The cost of this repeal will be £640,000 this year and £840,000 in a full year."

Earlier in the year the Proprietary Association of Great Britain had presented a Memorandum dealing with the problem to the Chancellor which indicated the basis of an agreement between the interested parties in the following terms:—

The Pharmaceutical Society of Great Britain, the National Pharmaceutical Union, the Scottish Pharmaceutical Federation, the Company Chemists' Association and the Wholesale Drug Trade Association will raise no objection to the repeal of the Medicine Stamp Acts if at the same time there is inserted in the Finance Act repealing these duties a provision which gives the pharmacist equivalent privileges in exchange for that which he now enjoys, and this has been embodied in a draft Bill which is Annexure No. 2 hereto. The Proprietary Association of Great Britain and some of the other parties concerned in these negotiations have agreed that the acceptance of Annexure No. 2 shall be coupled with an undertaking by the Proprietary Association of Great Britain to support a Bill to control the industry, which Bill has been prepared and agreed in principle. The Proprietary Association of Great Britain undertakes to support this Bill accordingly, and it is hoped that the appropriate Government Department will, when opportunity arises, also support this Bill and introduce it as a legislative measure.

On June 26th, 1941, the Government Bill was introduced. The Lord President of the Council, the Chancellor of the Exchequer, the Secretary of State for Scotland, the Attorney-General and

Mr. Peake, Under-Secretary, Home Office, supported the Bill which, in addition to repealing the stamp and licence duties, proposed to regulate the sale of medicines, to prohibit advertisements relating to certain medical matters and at the same time effect some minor amendments to the Pharmacy and Poisons Act, 1933. During the Committee stage of the Bill the following additional clauses were proposed, but the first was withdrawn and the second negatived on a vote:—

(a) No person shall sell or advertise under a fancy proprietary name any preparation having essentially the same composition as preparations listed in the current editions of the British Pharmacopœia or of the British Pharmaceutical Codex.

(b) No person other than a person authorised under sub-section (1) of section seven (section 12 of the Act) shall sell any article comprising a substance recommended as a medicine under a proprietary designation unless it was, at the time of the passing of this Act, being sold under that designation.

Royal Assent was given to the Bill on August 7th, 1941. The essential provisions as they affect the sale of medicines are:—

Advertisements. Advertisements relating to Bright's disease, cataract, diabetes, epilepsy or fits, glaucoma, locomotor ataxy, paralysis or tuberculosis in terms which are calculated to lead to the use of the articles for the purpose of the treatment of human beings are prohibited (Section 8). Advertisements published by certain public bodies and by voluntary hospitals are excluded from the prohibition. A defence is provided to allow publication of such advertisements to a restricted group of persons which includes registered medical practitioners, registered pharmacists, authorised sellers of poisons and nurses and those undergoing training for such registration. Permission has since been given by the Minister of Health, under powers conferred on him, "permitting the publication of any advertisement referring to insulin in terms which are calculated to lead to its use for the purpose of the treatment of human beings for diabetes, but not otherwise referring to any article, or articles of any description, in such terms as are mentioned in subsection 1, section 8, of the Pharmacy and Medicines Act, 1941." Advertisements referring to articles in terms which are calculated to lead to the use of the articles for procuring the miscarriage of women are prohibited by Section 9 of the Act, which does not contain the defence provided for the preceding section, although Section 10 provides a defence restricted to advertisements in technical publications.

Disclosure of Composition. Any substance which is referred to in terms which are calculated to lead to the use of the substance for the prevention or treatment of any ailment, infirmity or injury affecting the human body must have a quantitative disclosure of composition.

An exception is provided in the case of an article prepared for a particular person if it is prescribed for the needs of that person. This provision allows the supply of counter-prescribed medicines complying with the stated terms of exemption and consequently disclosure is not necessary in such cases.

Selection of Name. Misleading and unscientific terms must not be used. The Act requires the Poisons Rules procedure to be followed for those ingredients which are poisons. If not a poison the British Pharmacopœia or British Pharmaceutical Codex titles must be used where applicable. Either the English, full Latin, precise abbreviated form or synonym may be used, but not chemical formulæ.

Where the preceding methods cannot be applied the recognised and accepted scientific name or other name which gives a true description of the ingredient must be used.

Statement of Quantities. No quantity need be stated where the substance as a whole has an appropriate description as indicated above. The amount of an ingredient is to be stated as the approximate percentage or the approximate quantity in each container. An alternative is provided, e.g., in the case of tablets and powders, where the amount in each portion may be stated. Unless the article is a poison it is not necessary to state the number of "portions" in each package. The disclosure must be placed on the immediate container, e.g., bottle, box or each individual wrapper where single powders are supplied from bulk packings. An exception to the disclosure requirement is made if the substance is referred to in terms which are not "terms which give a definite indication that the substance is intended to be used as, or as part of, a food or drink, and not as, or as part of, a medicine."

Restriction on Sales. All retail sales of recommended medicines must be made from a shop and are restricted to certain classes of persons. The seller may be a doctor, dentist or authorised seller of poisons or, if not a member of one of these classes, one who has served a regular apprenticeship and on August 7th, 1941, was carrying on the retail sale of drugs on his own account with the business, so far as it concerns the sale of drugs, under his personal control. Sales by an authorised seller of poisons are restricted to premises registered under Part 1 of the Pharmacy and Poisons Act 1933. Sales of a dried, crushed or comminuted plant or a mixture of such plant or plants with water, also natural, or artificial imitations of natural mineral waters can be made by all traders provided the sale is made from a shop. A person not authorised as described above may sell proprietary medicines subject to certain limitations, the effect of which practically restricts the sale of non-proprietary recommended *B.P.* and *B.P.C.* preparations to the authorised class of sellers. The Act defines "proprietary designation" in terms which, in effect, bring the article within the scope of the Act if the seller describes it, or if the article is labelled, in such a way as to indicate the goods of a particular person.

FORMULÆ OF PROPRIETARY MEDICINES

Owing to the legislation which now makes compulsory the disclosure of formulæ on all preparations sold for medicinal use,

we are able to provide fuller information on the composition of so-called "patent medicines." The following list is composed of a representative selection of nationally advertised remedies supplied through the normal wholesale channels as being those in most frequent demand. In most cases, the name of the manufacturer is given together with the principal claims and uses of each preparation. *All information relating to both formulæ and uses is stated precisely in the manner in which it appears on the label of the container or in accompanying literature.* An indication of the recommended dosage is also given.

The disclosure of formulæ has abolished the principal criterion by which ethical remedies were formerly distinguished. Although the disclosure is frequently vague and sometimes misleading, to a degree sufficient to prevent the production of an exact imitation, secret remedies are to-day non-existent. For this reason the question as to whether a product is ethical or non-ethical, whether it should be described in Volume I or Volume II of the *Extra Pharmacopœia*, can no longer be determined by reference to the amount of information available as to its composition. A more practical discrimination is made possible by considering the method of distribution, i.e., whether a preparation is sold to the public by advertisement and without reference to the requirements of the individual patient or whether it is a product recommended or prescribed by doctors or pharmacists for the treatment of individual patients.

Adrax Tablets (*W. B. Cartwright Ltd., Rawdon*). "A scientifically devised double treatment restoring to the kidneys and bladder their normal functions. A cure for backache, bladder weakness, lumbago, rheumatism, acidity, bladder irritation." *Formula*:—*White tablets*:—Pot. Bicarb. gr. 14, Sod. Acet. gr. 11, Pot. Cit. gr. 11, Acid. Citric. gr. 0.7. *Brown tablets*:—Aloes gr. 1, Potas. Nitrates gr. 1, Asafoetida gr. 1, Methylthionin Hydroch. gr. $\frac{1}{2}$, Ol. Juniper min. $\frac{1}{2}$, Capsicum gr. $\frac{1}{2}$, Excipient q.s.

[P2] **Aero-Cotarol** (*Maw, Son & Sons, Barnet*). "A scientific germ-destroying vaporant of remarkable potency. For the treatment and prevention of influenza, bronchitis, asthma, catarrh, whooping cough and all respiratory disorders." *Formula*:—Contains 44.5% w/w of cresols.

Agarol Compound (*William R. Warner & Co. Ltd., London*). "A perfectly homogenised emulsion. Indicated in constipation." *Formula*: Active constituents: Paraff. Liq. 25% v/v, Phenolphthal. 1.3% w/v, Agar 0.2% w/v. *Dose*: $\frac{1}{2}$ to 1 tablespoonful. Children, $\frac{1}{2}$ to 2 teaspoonfuls.

Agocholine Soluble Granules (*Bengué & Co. Ltd., Wembley*). "A valuable remedy in jaundice, congestion of the liver and gall bladder, and subacute and chronic cholecystitis whether stones are present or not. Relieves the local and general symptoms of hepatic origin, such as nausea, vertigo, migraine, urticaria, anorexia and constipation due to biliary insufficiency." *Formula*:—Approx. Percentages, Peptone 6.2, Mag. Sulph. 31.4, Saccharum Alb. 62.4. *Dose*: 1 to 3 teaspoonfuls.

Agonsair (*Agonsair Co. Ltd., London*). "For destroying nits and vermin. Harmless in nature." *Formula*:—Active Ingredients: Quassia Lignum 7.0%, Ol. Rosmarini 0.5%, Sod. Borate 1.5%, Spt. Meth. Indust. 15.0%, Ext. Quassia 0.5%.

Alasil Brand (*A. Wander Ltd., London*). "For rheumatism, influenza, colds, headache, neuralgia, toothache and for relieving pain generally." *Formula*:—5 grs. Acetyl-Salicylic Acid, 6 grs. Alocol (Colloidal Hydroxide of Aluminium) and $1\frac{1}{2}$ grs. of Calcium Phosphate Bibasic.

Alka-Zane (*W. R. Warner & Co. Ltd., London*). "Alkalizer, antacid, diuretic. Alka-Zane promotes the elimination of those acid products of metabolism, the excessive formation and retention of which in the body may

lead to acidosis, acidæmia and similar conditions." *Formula*:—Sod. Bicarb. 48%, Pot. Bicarb. 7%, Ac. Cit. 24%, Soda Cit. 12%, Calc. Phosph. 3.5%, Mag. Phosph. 3.5%, Calc. Glycerophosph. 1.6%. *Dose*: 1 heaped teaspoonful.

Alkia Saltrates (*Saltrates Ltd., London*). "A positive and natural corrective for stomach, blood, liver, kidney, bladder and intestinal disorders. Unrivalled as a specific for rheumatism, catarrhal affections, dyspepsia, gout, etc." *Formula*:—Magnes. Sulph. Exsicc. 27.57%, Sodii Sulph. Exsicc. 25.73%, Sodii Chlorid. 1.38%, Lithii Carbonas. 0.69%, Saccharin 0.06%, Effervescent base to 100.00%. *Dose*: 1 to 3 teaspoonfuls.

Allcock Brand Porous Plaster (made in U.S.A.; European agency:—*The Allcock Manufacturing Company, Birkenhead*). For rheumatism, neuritis, etc. Rubber 43.39%, Burgundy Pitch 27.14%, Frankincense 20.25%, Orris Root 8.70%, Capsicum 0.27%, Beeswax 0.18%, Camphor 0.04%, Gum Elemi 0.02%, Gum Myrrh 0.01%.

[P1] **Allophen** (*Parke, Davis & Co., London*). Chocolate-coated pills. *Formula*:—Aloin $\frac{1}{2}$ gr., Phenolphthalein $\frac{1}{2}$ gr., Strychnine $\frac{1}{10}$ gr., Ext. Belladonna B.P. $\frac{1}{2}$ gr., Powd. Ipecacuanha $\frac{1}{2}$ gr. *Dose*: 1 to 3 pills.

Aludrox Brand Amphoteric Gel (*John Wyeth & Brother Ltd., London*). "Acts as an amphoteric colloid in the removal of hydrochloric acid from the stomach." *Formula*:—Aluminium hydroxide 6.0%, Sodium benzoate 0.5%, Oil of Peppermint 0.01%. Distilled Water 9.5%. *Dose*: 1 or 2 teaspoonfuls.

Anaclasine Brand Tablets (*Bengué & Co. Ltd., London*). For anaphylactic states of alimentary origin. One tablet contains Hydrated Magnesium Hyposulphite 0.15 g., Hydrated Sodium Hyposulphite 0.03 g., Magnesium Silicate 0.02 g., Hydrated Magnesium Bromide 0.01 g., Meat Peptones (beef, mutton, horseflesh, pork and game) 0.02 g., Fish and Crustacean Peptones (cod, whiting, gurnet and lobster) 0.01 g., Whole Egg Peptone 0.0025 g., Milk Peptone 0.005 g., Vegetable Peptones (peas, beans, lentils and cereals) 0.01 g., Peptone of Animal Gelatine 0.0025 g.

Anadin Brand Tablets (*Anadin Ltd., London*). "For relief of pain, headache, neuralgia, neuritis, colds, rheumatism, periodic pains and for pain following tooth extraction." *Formula*:—Each tablet contains acetphenetidin. 2 gr., acetylsalicylic acid 4 gr., caffeine alkaloid $\frac{1}{2}$ gr. *Dose*: Adults, 1 or 2 tablets; children, $\frac{1}{2}$ to the adult dose.

Analax (*Analax Works, London*). "A delicious fruit laxative pastille." Seaweed 4%, Citric Acid 1.5%, Phenolphthalein 9%, Sugar 63%. *Dose*: 1 or 2 pastilles.

Andrews Liver Salts (*Scott & Turner, Newcastle-on-Tyne*). "A pleasant-tasting effervescent saline which taken regularly helps to maintain perfect fitness by cleansing the body from accumulations of poisonous waste." *Formula*:—Acid. Tart. and/or Cit. 21 to 25%, Sod. Bicarb. 23 to 27%, Mag. Sulph. 17 to 21%, other constituents 31 to 35%. *Dose*: 1 or 2 teaspoonfuls in a glass of water.

[P1] **Anestan Asthma Tablets** (*Anestan Ltd., London*). "A specialist's infallible prescription for asthma, bronchitis, bronchial asthma, hay fevers." *Formula*:—Active constituents: Fluoresc. Solub. 1.5%, Calc. Glucon. 1.0%, Theophyll. 2.0%, Ephed. 4.9%, Phenazon. 24.0%, Theobrom. 10.0%. *Dose*: 1 to 2 tablets. Children $\frac{1}{2}$ to 1 tablet.

[P1] **Anestan Brand Ointment** (*Anestan Ltd., London*). "A miraculous healing ointment for eczema (all types) bad legs, abscesses, ring-worm, carbuncles and boils, running sores, acne." *Formula*:—Titanii Salicyl. 0.31, Hydrarg. Oxid. Flav. 0.31% w/w, Titanii Borat. 5.00, Flores Zinci 28.10, Adipis Suilli 33.14, Adipis Lanæ Hyd. 33.14, Liq. Azorubri q.s., Perfume q.s.

Antexema (*The Antexema Company, London*). "For eczema, insect bites, acne, chilblains, chafed skin and similar skin troubles of babies and adults." *Formula*:—Petroleum jelly 35.4, Gum Acacia 12.4, Boric Acid 1.5, Almond Oil or Nut Oil 1.5, Water 49.2.

[P1] **AntiKamnia Brand Tablets** (*The AntiKamnia Remedy Co., Fassett & Johnson, London*). "For headache, neuralgias, toothaches, rheumatic and sciatic pains, colds, women's pains, all pains and aches." *Formula*:—Acetanilide 3.000 grs., Caffeine Citrate 0.475 grs., Soda Bicarbonate 1.525 grs., excipients q.s. *Dose*: 1 or 2 tablets.

Antiphlogistine Brand (*Denver Chemical Mfg. Co., London*). "For inflammation and congestion." *Formula*:—Sodium Lactate 43.500%, Acid Boricum 00.100%, Acid Salicylicum 00.020%, Menthæ Ess. 00.002%, Menthyl Ess. 00.002%, Eucalypti Ess. 00.002%, Kaolin 56.374%.

Anusol Hæmorrhoidal Suppositories (*William R. Warner & Co., London*). *Formula*:—Bism. Subgall. 2%, Bism. Resorcinate 1.75%, Bism. Subiod. 0.07%, Zinc. Oxid. 10.5%, Acid. Boric. 18%, Bals. Peruv. 2.8%.

Archanium (*The Phenolaine Company, Goudhurst*). "For rheumatism, backache, neuritis and fevers." *Formula*:—Sodium Methyl Carboxyl Salicylate 90%, Sodium Bicarb. 10%, Traces of Potass., Bicarb., Sulphate and Chloride from Willow Ash. *Dose*: $\frac{1}{2}$ teaspoonful.

Ashton and Parsons Infants' Powders (*Phosferine (Ashton and Parsons) Ltd., Watford*). "For children teething and for slight stomaclic derangement, incidental to infancy." *Formula*:—Tincture of Matricaria (1 in 10) 3.12%, Lactose 96.88%. *Dose*: Under six months, half a powder; above six months, one powder.

Askit Powders (*Askit Ltd., Glasgow*). "The scientific cure for bilious and nervous headaches—influenza, neuralgia, rheumatic and all nerve pains." *Formula*:—This powder contains Acidum Acetylsalicylicum 0.6 gm., Acetphenetidin 0.49 gm., Caffeinæ Citras 0.125 gm., Magnesii Trisilicas 0.015 gm. *Dose*: 1 powder.

Askit Tablets (*Askit Ltd., Glasgow*). "Cures headaches and all nerve pains." *Formula*:—Acidum Acetylsalicylicum 2.375 gr., Acetphenetidin 1.85 gr., Caffeinæ Citras 0.475 gr., Magnesii Trisilicas 0.05 gr. *Dose*: 2 to 4 tablets.

[P1] **Asmadyn** (*John Wyeth & Bro., London*). "To control spasms and reduce severity and frequency of attacks in asthma and hay fever." *Formula*:—Phenazone gr. 1, Ephed. Hydr. Synth. gr. $\frac{1}{2}$, Dimethylxanthine gr. $\frac{1}{2}$, Trimethylxanthine gr. $\frac{1}{2}$, Grindel. Camp., gr. 1, Potass. Brom. gr. 2. *Dose*: 1 or 2 tablets.

[P1] **Asmolin Brand Tablets** (*D. Mawdsley & Co., Manchester*). "For asthma, catarrh, bronchitis." *Formula*:—Fluoresc. Solub. 0.0015 grammes, Ephed. Hydrochlor. B.P. 0.02 grammes, Caffein 0.032 grammes, Theobrom. 0.041 grammes, Calcii Gluconas 0.041 grammes, Base to 0.324 grammes. *Dose*: 1 tablet.

Aspro (*Aspro Ltd., Slough*). "Speedily dispels headaches, nerve pains, nerve strain, colds, influenza, rheumatic pains, etc." *Formula*:—Each of these tablets contains 5 grains purest Acetylsalicylic Acid.

Asthmasan Brand Inhalant (*Francis Riddell Ltd., London*). "Composition: Adrenalin 0.12%, Ephedrine 0.03%, Carbinyl-paraoxybenzoate 0.2%, Glycerine and Water ad 100%."

Atkinson and Barker's Infants' Preservative (*Robert Barker & Son Ltd., Manchester*). "For disorders incident to infants and for allaying the pains attendant on the cutting of their teeth, etc." *Formula*:—Magnes. Carb. 5%, Sod. Bicarb. 1.5, Sugar 9.75, Alcohol 7.0, Sweet Spirit Nitre 0.625, Saffron 0.05, Oils of Dill and Fennel 0.16.

Backache and Kidney Pills (*The Mackenzie Medicine Co. Ltd., London*). "These pills will give relief in the various complaints resulting from disordered kidneys and bladder." *Formula*:—Powd. Buchu 1 gr., Oil Juniper 1.2 gtt., Uve Ursi 1 gr., Potass. Nitrate aa 1 gr., Podophyllin 1.20 gr. *Dose*: 1 to 3 pills.

Balmosa (*Oppenheimer, Son & Co. Ltd., London*). "The methyl salicylate in Balmosa is very rapidly absorbed and exhibits in a remarkable degree the characteristic effects of the salicylates in rheumatism, muscle and joint pains, neuralgia, sciatica, tarticollis, etc." *Formula*:—Methyl Salicylate 4%, Menthol 2%, Camphor 4%, Capsicum 0.3%.

Balsamic Emulsion (*Pharmico Laboratories, Derby*). "The tonic remedy for the cure and prevention of coughs, colds, catarrh, etc." *Formula*:—"Approx. percentages of active constituents, Paraff. Liq. 25, Calc. Hypophosph. 78, Sod. Hypophosph. 78, Camphor 0.2, Ol. Anisi 34, Acid Benzoic 17, Tinct. Benzoin Co. 1.56, Sp. Chlorof. 47, Chlorof. 34, Ol. Menth. Pip. 0.5, Tinct. Capsici 0.1. *Dose*: 1 dessertspoonful. Children, 1 teaspoonful.

Barker's Liquid of Life (*Professor Barker, Manchester*). "For indigestion, dyspepsia, biliousness, constipation, jaundice, enlargement of liver, stomach, liver and kidney troubles. All chronic diseases such as rheumatism, dropsy, fevers, ulcers and bronchitis." *Formula*:—Quassia 7%, Gentian 7%, Burdock 4%, Chillies $\frac{1}{2}$ %, Ginger 1%, Rhubarb 1%, Peru Bark 1%, Columba Root 2%, Aloes 8%, Soda Bi-carb. 13%, Syrup 55%. *Dose*: Half to a teaspoonful. Children, half quantity.

Bates and Co's Compound Breast Salve (*Bates & Co., London*). "This salve is a medicinal preparation for external use only in cases of wounds and sores. Its use as a breast salve is confined to cases of simple inflamed breasts, upon which it acts as a drawing and healing salve." *Formula*:—Ol. Rap. 20.4, Coloph. 46.1, Cera Flav. 29.3, Ol. Terebinth. 1.1, Ol. Thym. 0.5, Zinc. Carb. 2.6.

Baxen Brand Powders (*E. Griffiths Hughes Ltd., Manchester*). "Quickest safe relief from pain. For headaches, neuralgia, backache, periodic pains of women, neuritis, rheumatic pains, sciatica and for feverish conditions, 'flu, sleeplessness, etc.'" *Formula*.—In one powder: Acetparaphenolide 0.2916 gm., Theobromine 0.0216 gm., Phenazonum 0.1296 gm., Theine 0.0432 gm., Potass. Sulph. 0.0324 gm., Ferri Ox. Calc. 0.0008 gm. *Dose*: For adult, one powder.

Baxen Brand Tablets (*E. Griffiths Hughes Ltd., Manchester*). "Give quickest and long lasting relief from pain. For headaches, period pains, neuralgia, backache, neuritis, sciatica, rheumatic pains, sleeplessness, feverish conditions, 'flu, etc.'" *Formula*. Per tablet—Acetphenetidinum 0.1458 gm., Phenazonum 0.0648 gm., Theobromina 0.0108 gm., Caffeina 0.0216 gm., Ferri Oxid. 0.0003 gm., Dextrosium 0.0259 gm., Amylum 0.0421 gm., Creta Gall. Pur. 0.0065 gm. *Dose*: 2 tablets.

Beecham's Cough Pills (*Beecham's Pills Ltd., St. Helens*). "Represent a simple and safe remedy for bronchial affections, hoarseness, shortness of breath, tightness and oppression of the chest, wheezing and coughs in general." *Formula*.—Camphor 4.52%, Terpin. Hydr. 5.64%, Pulv. Scill. 4.24%, Ext. Glycyrrhiz. Pulv. 28.32%, Pulv. Glycyrrh. 19.20%, Pulv. Seneg. 8.48%, Mag. Carb. Lev. 2.80%, Ext. Lactuc. 16.97%, Ol. Anis. 1.21%, Ol. Abiet. 2.22%, Acaciæ Gummi 6.40%. *Dose*: 3 to 6 pills.

Beecham's Lung Syrup (*Beecham's Pills Ltd., St. Helens*). "For coughs, colds, bronchitis, throat and chest ailments." *Formula*.—Ext. Scill. Liq. 0.94%, Tinct. Capsici 0.05%, Olea Essentia (Menth. Pip., Anis., Limon., Caryoph.) 0.07%, Sodii Benzoas 0.91%, Ammonii Bromidum 0.46%, Tinct. Bryoniæ 0.31%, Chlorof. 0.38%, Sp. Chlorof. 0.55%, Lævulosum 19.12%, Dextrosium 19.12%, Sacrosium 28.78%, Infusum Senegæ ad 100.00. *Dose*: Adults, 2 teaspoonfuls. Children $\frac{1}{2}$ to 1 teaspoonful.

Beecham's Pills (*Beecham's Pills Ltd., St. Helens*). "For constipation, sick headache, biliousness, indigestion." *Formula*.—Pulvis Zingiberis 26.38%, Pulvis Coriandri 5.67%, Sapo Purus 10.65%, Aloe 54.56%, Oleum Juniperi 0.89%, Oleum Rosmarini 0.9%, Oleum Anisi 0.23%, Oleoresina Capsici 0.12%, Oleoresina Zingiberis 0.60%. *Dose*: 2 or more pills.

Beecham's Powders (*Beecham's Pills Ltd., St. Helens*). "For influenza colds, headache, neuralgia, rheumatism and nerve pains." *Formula*.—Acetphenetidin B.P. 30.00%, Acidum Acetylsalicylicum B.P. 38.00%, Caffeina B.P. 1.62%, Kaolinum B.P. 27.83%, Oleum Cinnamomi 2.08%, Saccharinum Solubile B.P. 0.47%. *Dose*: 1 powder.

Beecham's Powders (Tablet form) (*Beecham's Pills Ltd., St. Helens*). "For colds, headaches and nerve pains. A splendid tonic at any time." *Formula*.—Acidum Acetylsalicylicum 46.13%, Acetphenetidin 36.42%, Caffeina 1.97%, Oleum Cinnamomi 0.95%, Saccharinum Solubile 0.57%, Acaciæ Gummi 1.21%, Amylum 5.47%, Creta Gallica Purificata 6.37%, Acidum Stearicum 0.91%. *Dose*: Two tablets equal one powder.

Bell's (John) Sea and Travel Sickness Remedy (*John Bell, Hills and Lucas Ltd., London*). *Formula*.—Each capsule contains 5 grains Chlorbutol. *Dose*: 1 capsule.

Beltona Brand Antiuritic Tablets (*Beltona Ltd., Hoddesdon*). "For arthritis, rheumatism, neuritis, lumbago, sciatica, and all nerve pains." *Formula*.—Each of these tablets contains—Sulphur Sublimatum 2.00%, Amylum Solanum 24.60%, Acacia Gummi 16%, Liq. Bixa Orellana 7%, Ortho Aceto Oxybenzoic acid. 36.55%, Sol. Rubra Dulcis 2.30%, Tri-Methyl Xanthine Benzoas 8.34%, Lactosum Pur. 25.00%, Ft. Tab. grs. 5 *Dose*: 2 tablets.

Beltona Brand Lotion (*Beltona Ltd., Hoddesdon*). "For rheumatoid arthritis, rheumatism, lumbago, neuritis, gout, sciatica, sprains, neuralgia, and all inflammatory conditions." *Formula*.—This preparation contains Liquid Ammonia 3.3, Oleum Citronellæ 3, Oleum Verbenæ 1, Methyl Salicylas 3, Castor Oil 9, Alcohol (Industrial) 94.8, Colouring 3.

Beltona Brand Ointment (*Beltona Ltd., Hoddesdon*). "To be applied after using Beltona Brand Lotion. Also for cuts, sores, abrasions, chapped hands and any irritation." *Formula*.—Monohydroxysterol 1.0, Ozokerite 10.0, Hydrocarbon base 39.0, Glycerol 1.0, Aqua destillata 49.0.

Bengué Brand Balsam (*Bengué & Co. Ltd., Alporton*). "Gout, rheumatism, lumbago, sciatica, neuralgia, neuritis." *Formula*.—Approximate percentages: Menthol 20%, Methyl Salicyl 20%, Cera Flav. 6%, Adeps Lanæ 54%.

Benzedrine Inhaler (*Menley & James Ltd., London*). "For relieving nasal congestion in head colds, sinusitis, hay fever, asthma, rhinitis, and nasal catarrh." *Formula*:—Each tube is packed with β aminoisopropylbenzene (amphetamine) 0.325 gm., oil of lavender 0.097 gm., and menthol 0.032 gm.

Besorbon Medicinal Snuff (*Kensales Ltd., London*). "For colds and catarrh, headaches and hay fever." *Formula*:—Soda Bicarb. 1.380%, Bismuth Carb. 8.849%, Mag. Carb. Pond. 35.309%, Acid Boric 8.849%, Menthol 4.424%, Perfumed Base 41.189%.

B.F.I. Antiseptic (*Sharp & Dohme Ltd., London*). "An effective first-aid dressing for cuts and scratches. Relieves aching feet. Promotes healing and soothes chafing, sunburn and skin irritations. Useful after shaving." *Formula*:—Each ounce of the powder contains: Bismuth-Formic-Iodide 70 gr., Zinc Sulphocarbonate 10 gr., Bismuth Subgallate 20 gr., Powdered Alum 3 gr., Boric Acid 128 gr.

Bilax (*Foster McClellan Co., London*). "Doan's Dinner Pills. Recommended for biliousness, constipation, torpid liver, indigestion." *Formula*:—Aloin 16.667%, Podophyllin 13.333%, Leptand. 8.333%, Jalap. Res. 8.333%, Fruct. Capsic. 2.083%, Ol. Menth. P. 1.333%, Oleores Zingib. 4.445%. *Dose*: 1 to 4 pills.

Bile Beans (*C. E. Fulford Ltd., Leeds*). "For biliousness and constipation, headache, indigestion, impure blood, sallowness, dizziness, bad breath, debility and other liver and stomach troubles, also rheumatism and obesity." *Formula*:—Aloes Barb. 6.67%, Res. Podoph. 4.42%, Res. Scammon. 8.85%, Leptandrin 3.30%, Pulv. Ext. Jalap. 8.85%, Pulv. Ext. Coloc. 2.67%, Ext. Gentian 17.78%, Cascarin 6.67%, Cardam. 2.67%, Zingib. 10.67%, Saponis Cast. 1.65%, Ol. Menth. Pip. 4.42%, Ol. Res. Capsici 0.75%, Ol. Res. Zingib. 4.42%, Excipient ad 100.00%. *Dose*: 1 or 2 pills.

Bilson's Laxative Cleanser (*T. J. Bilson & Co., London*). "A herbal remedy for constipation which cleanses and purifies the system in a natural manner." *Formula*:—Composed of equal parts of fennel, mate. *Dose*: 1 level teaspoonful.

Birley's Antacid Powder (*Birley's Antacid Ltd., London*). "Instant neutraliser of excess acid." *Formula*:— $3\text{MgCO}_3 \cdot \text{Mg}(\text{OH})_2 \cdot 4\text{H}_2\text{O}$ approximating to B.P. Codex:—Mag. Carb. Lev. 100%.

Bishop's Natural Fruit Saline (*Alfred Bishop Ltd., London*). "This preparation is an excellent remedy in all stomach and liver troubles, neutralising and washing out all those waste products of digestion which are the cause of all ordinary ailments, such as indigestion, acidity, headache, giddiness, pain and aching in the right side, etc." *Formula*:—Sodii Bicarbonas 43.49%, Acidum Citricum 52.17%, Magnes. Sulphas. 4.34%. *Dose*: 1 teaspoonful.

Bishop's Varalettes (*Alfred Bishop Ltd., London*). "For gout, rheumatism, gravel, eczema, sciatica, etc." *Formula*:—Lithii Carbonas 10.40%, Lithii Citras 7.42%, Sodii Bicarbonas 21.78%, Acidum Tartaricum 20.80%, Acidum Citricum 39.60%. *Dose*: 1 tablet dissolved in water.

Bishop's Varalettes Vichy Salts (*Alfred Bishop Ltd., London*). "Useful in kidney and liver troubles, diabetes and gout." Made and produced in England from the analysis of the natural water. The composition of this preparation was not disclosed. *Dose*: 2 tablets dissolved in water.

Bisodol (*Bisodol Ltd., London*). "Invaluable for indigestion, dyspepsia, heartburn, flatulence, acidity, etc." *Formula*:—magnesi carbonatis levis 39.27, sodii bicarbonatis 46.09, bismuthi subnitrat, papaini, diastasi aa 1.40, olei menthae piperitæ 0.44. *Dose*: 1 teaspoonful.

Bisodol Mints (*Bisodol Ltd., London*). "Exceedingly effective in cases of acidity, flatulence, heartburn, sourness and nausea, and all forms of indigestion." *Formula*:—Each 11 gr. tablet contains: magnesi hydroxidi 2.01, calci carbonatis 2.51, sodii bicarbonatis 2.01, bismuthi carbonatis 0.51, papaini, diastasi aa 0.10, sucrosi 2.51, saccharini solub. 0.05, amyli 0.82, calci stearatis 0.11, talci 0.09, ammonii carbonatis 0.12, olei menthae piperitæ 0.06. *Dose*: 2 to 4 tablets.

Bis-U-Mint Ovals (*Gold Seal Proprietaries Ltd., Croydon*). "World's best indigestion tablets. For acidity, wind, flatulence, etc." *Formula*:—Bis. Carb., Mag. Carb., Soda Bicarb., Oil of Peppermint, Oil of Cardamoms, Oil of Orange.

Bisurated Magnesia (*Bismag Ltd., London*). "For indigestion, dyspepsia, heartburn, gastritis, acidity, wind, etc." *Formula*:—Bism. Carb. 8%, Sod. Bicarb. 42%, Mag. Carb. 50%. *Dose*: $\frac{1}{2}$ to 1 teaspoonful.

Bisurated Magnesia Tablets (*Bismag Ltd., London*). "For all forms of digestive and stomach trouble." *Formula*:—Sodii Bicarb. 35.80, Mag. Carb. 42.62, Bis. Carb. 6.83, Excip. to 100. *Dose*: 2 to 4 tablets.

Bisuroids Laxative Tablets (*Bismag Ltd., London*). "These pleasant chocolate-flavoured tablets stimulate bowel lubrication and restore natural muscular activity; they do not gripe or force." *Formula*:—Containing Phenolphthalein 36·36%, Sucrose 36·36%, Theobroma Paste 27·28%. *Dose*: 2 to 4 tablets. For children, 1 tablet.

[P1] **Blair's Brand Gout and Rheumatic Pills** (*Prout & Harsant Ltd., London*). "Immediate relief for the sufferer. Not only cure—but prevent recurrence. For gout and rheumatic gout. For suppressed gout, including gouty asthma, bronchitis, dyspepsia, rheumatism, rheumatic headaches, lumbago, tic douloureux, pains in the head, face, etc." *Formula*:—Each pill contains Colchici Cormus B.P. 1·94 gr., Alumen Exsiccatum 0·46 gr., Excipient ad 3·0 gr. *Dose*: 2 pills.

Blanchard's Female Pills (*Leslie Martyn Ltd., London*). "For disorders and irregularities of the female constitution." *Formula*:—Ferrous Sulph. Exsicc. 10%, Pulv. Rhei 20%, Pulv. Cinnamon. 12%, Pulv. Cardamom. 12%, Pulv. Zingib. 12%, Syrup of Glucose 30%, Apioi 4%. *Dose*: 2 pills.

Blinblow Asthma Cure (*The manufacturer's name is not given on the container*). "A most effectual remedy for asthma, bronchitis, hay fever, influenza, whooping cough, etc." *Formula*:—Powdered Stramonium 80%, Potassium Nitrate 18%, Potassium Chlorate 1%, Eucalyptus Oil 1%. This preparation contains 0·30 per cent. (approx.) of Daturine.

Blinblow's Eucalyptus and Stramonium Cigarettes (*The manufacturer's name is not given on the container*). "For asthma, hay fever, influenza." *Formula*:—Stramonium Leaf 87%, Potassium Nitrate 12%, Eucalyptus Oil 1%.

[P1] **Blue Lion Fox Nuts** (*Shadforth Prescription Service Ltd., London*). "Heart, stomach, kidney and nerve tonic." *Formula*:—Dry Extract of Nux Vomica B.P. 1/16th grain, Powdered Digitalis Leaves B.P. 1/175 grain, Gentian Extract 1 grain, Dandelion Extract 1 grain. *Dose*: 1 to 3 pills.

Bonomint Laxative Chewing Gum (*Westminster Laboratories Ltd., London*). "Ends constipation quickly, and because it is harmless and not habit forming it is readily taken for ailments resulting from constipation such as headache, depression, irritability, tired feeling, bad complexion." *Formula*:—Saccharum Purificatum 72·95%, Glucosum Liquidum 10·19%, Phenolphthaleinum 5·16%, Acaciæ Gummi 1·09%, Oleum Menthæ Piperitæ 0·36%, Latex of Achras Sapota 10·25%. *Dose*: 1 or 2 tablets. Children 1/2 to 1 tablet.

Bowden Brand Indian Balm (*Bowden Indian Balm Co., Barnstable*). "For muscular complaints, skin diseases, burns, eczema, piles, cuts, chilblains and rheumatism." *Formula*:—Ung. Althæ 6%, Ol. Palmæ 4%, Ol. Chaulmoogræ 5%, Ol. Olivæ 6%, Ol. Ricini 6%, Ol. Camph. Ess. 1%, Ol. Cajaputi 1·25%, Ol. Rosmarini 1·25%, Ol. Terebene 1·25%, Ol. Orig. Alb. 1·25%, Ol. Eucalypti 1·25%, Camphora 5%, Lin. Saponis 1·25%, Bals. Canad. 5%, Ointment Base 68%.

[P1] **Bow's (Dr.) Liniment** (*Dr. Bow's Liniment Ltd., Edinburgh*). "A certain cure for coughs, colds, croup, bronchitis, rheumatism, lumbago, sprains." *Formula*:—Contains Ext. Papaveris Somif. Morphine 0·18%, Ext. Belladonnæ Virid. B.P.C. 1%, Flor Camp. 6·15%, Liq. Ammon. Fortis. 0·96%, Ext. Color 1·92%, Spt. Methyлатus Indust. 89·79%.

Box's Herbal Ointment (*W. H. Box, Plymouth*). *Formula*:—P. Ulm. Fulv. 10·5%, P. Alth. 10·5%, Paraff. Moll. Flav. ad 100·0%.

Box's Indigestion Pills (*Giant Pill Manufactory, Plymouth*). *Formula*:—P. Myrrh. 18·02 p.c., P. Aloes 18·92 p.c., P. Ginger 18·02 p.c., P. Capsicum 18·02 p.c., P. Gentian 18·02 p.c., Ol. Cajaputi 2·70 p.c., P. Acacia 6·30 p.c. *Dose*: 2 pills.

Bragg's Charcoal Biscuits (*J. L. Bragg Ltd., London*). "Highly recommended by the faculty as an article of diet to persons suffering from indigestion, acidity, colitis, etc." *Formula*:—Each biscuit contains 12½% of Carbo Ligni.

Bragg's Charcoal Tablets (*J. L. Bragg Ltd., London*). "For indigestion, flatulence, acidity, colitis, etc." *Formula*:—Carbo Ligni 90%, excipient 10%. *Dose*: 2 or more tablets.

Bragg's Prepared Vegetable Charcoal (*J. L. Bragg Ltd., London*). "Indispensable in the treatment of indigestion, flatulence, abdominal pain, poisoning by arsenic, lead or aluminium and bacterial infection." *Formula*:—Pure carbon from selected young willow.

British Spa Crystal Salts (*Lord Bros. & Co. Ltd., Broadheath*). "Natural cure for rheumatism. Highly recommended by the Medical Faculty in all cases of rheumatism, gout, sciatica, neuritis, lumbago, constipation, obesity,

dyspepsia, indigestion and all disorders arising from the liver and kidneys." *Formula*:—Sod. Sulph. Cryst. B.P. Pur. *Dose*: $\frac{1}{4}$ to $\frac{1}{2}$ teaspoonful.

[P1] **Brocast Inhalant** (*Cecil Clayton Ltd., London*). *Formula*:—Hyoscyamin Hydrobromide 0.145%, Procain Hydrochloride 0.09%, Active Principle of Suprarenal Gland (Medulla) 0.2, Chlorbutol 0.7, Glycerin 33%, Calcium gluconate 0.15%, Sorbionic Acid Lactone 0.025, Sorbitol 0.3. (All ingredients w/v) Aqua to 100.

[P1-81] **Bromidia** (*Roberts & Co., London*). *Formula*:—Alcohol 10%. Each ounce contains Chloral Hydrate (B.P.) 91 Grs., Bromide of Pot. (B.P.), 91 Grs., Ext. Cannabis (B.P.C.) 1 Gr., Ext. Hyoscyamus Sicc. (B.P.) 1 Gr. *Dose*: $\frac{1}{2}$ to 1 teaspoonful.

Bronamalt (*Fletcher, Fletcher & Co. Ltd., London*). "The ideal tonic nutrient. Is not a medicine, but an agreeable tonic nutrient, actively assisting digestion, and improving nutrition." *Formula*:—Extract Malt 87 p.c.; Alcohol 9.5 p.c., Ext. Cinch. Liq. 2.0 p.c. (combined with Bromine). *Dose*: 2 teaspoonfuls; Children, 1 teaspoonful.

Brooklax Chocolate Laxative (*Westminster Laboratories Ltd., London*). "For constipation." *Formula*:—Chocolate 90%, Phenolphthalein 10%. *Dose*: 1 tablet; Children $\frac{1}{2}$ tablet.

[P1] **Brovon** (*Moore Medicinal Products, Aberdeen*). "The preparation of choice for dealing with all severe attacks of asthma." *Formula*:—Methyl Atropine Nitrate 0.14% w/v, Epinephrine 0.50% w/v, Chlorbutol 0.50% w/v, Pituitary Extract B.P. 1.00% w/v.

Bunter's Nerveine (*Bedford Laboratory, London*). "Cures toothache instantly. Gives permanent relief by painless constriction of the nerve in decayed teeth, forms a stopping, arrests decay and saves extraction. As a nerve tonic it gives strength to the nervous system." *Formula*:—Acid. Tann. 4.25%, Acid. Benz. 0.5%, Camph. 4.25%, Chlorof. 9.8%. *Dose*: 10 drops.

Burdock Pills (*G. E. Thompson & Co., Swansea*). "The great blood purifier. In whatever form impurity of the blood shows itself Burdock Pills will soon effect a cure." *Formula*:—Aloe Socot., Aloe Barb., Zingiber, Rheum, Sapo Castil., Cambogia, Capsicum, Burdock, Oleum Core Ang., Mag. Carbs. Lewis.

Burgess Lion Brand Pills (*E. Burgess Ltd., London*). "Particularly recommended for headache, giddiness, pains in the back or loins, loss of appetite, general debility." *Formula*:—P. Rhei. 24%, P. Ext. Aloes 18%, P. Sapo 12%, P. Myrrhæ. 12%, Ol. Menth. Pip. 2%, Syrup Excip. *Dose*: 2 pills.

Burgess's Lion Ointment (*E. Burgess Ltd., London*). "Its application is particularly recommended for boils, carbuncles, whitlows, poisoned wounds, abscesses, ulcers and bad legs, eczema, ringworm, itch, sycosis (Barber's Rash) impetigo, polypus, piles, fistula, chilblains, cuts, burns, scalds, bites, stings." *Formula*:—Adep. Lanæ Anhyd. 28%, Paraffin Mollé 28%, Cera Flav. 12%, Resina 12%, Zinc Oleas 12%, Sp. Vin. Methyl 8%.

Buxton Rubbing Bottle (*Braddock & Bagshaw Ltd., Oldham*). "Acting directly at the seat of the pain, it cures rheumatism, lumbago, sciatica and kindred ailments. Invaluable for sprains, stiff joints and unbroken chilblains." *Formula*:—Ext. Capsici 5.19, Spt. Tereb. Rect. 10.39, Camphoræ Sub. 6.92, Ol. Citronella 1.30, Ol. Essent. 1.30, Paraffinum ad 100.

[P1] **Caapi** (*H. R. Napp, London*). "Anti-coryza Tablets." *Formula*:—Atropine Sulphate $\frac{1}{100}$ gr., Caffeine 1 gr., Phenacetin 2 gr., Quinine Alkaloid $\frac{1}{2}$ gr., Cinnamon Pulvis 1 gr. *Dose*: 2 tablets.

Cadum Ointment (*Cadum Ltd., London*). "For eczema, psoriasis, pimples, eruptions, sores, skin irritation, cuts, scaly skin, burns, insect bites, itching piles, ringworm, shingles, chafing, complexion blemishes, rash, and other skin troubles." *Formula*:—Petrolatum 74.0%, Oxide of Zinc 15.0%, Sulphur 6.0%, Cade Oil 4.0%, Acid Salicylic 0.75%, Salicylate Methyl 0.25%.

Cal-Bis-Nate (*William R. Warner & Co. Ltd., London*). "Indications: sour stomach, flatulence, excessive acid secretion, heartburn, acid dyspepsia; in the treatment of gastric ulcer and gastric distress of any nature due to an excess of acid." *Formula*:—Mag. Carb. Lev. 16.30%, Sod. Bicarb. 32.70%, Calc. Carb. 27.10%, Colloidal Kaolin 10.80%, Bism. Subgall 2.30%, Ol. Menth. Pip. 0.07%, Bism. Carb. 5.20%. *Dose*: 1 level teaspoonful.

Calcium-D. (*Thos. Kerfoot & Co. Ltd., Vale of Bardsey*). "Presents the essential element Calcium in its most efficient form, together with natural Vitamin D to ensure assimilation." *Composition*:—Each teaspoonful contains approximately 17 gr. of Calcium Sodium Lactate with 500 International Units

of Vitamin D in a special granulated sucrose base, flavoured with Oil of Lime. *Dose:* 1 teaspoonful.

Caleno Brand Stabilized Calcium Aspirin (*J. C. Eno Ltd., London*). "For use in cases of headaches, toothache, neuralgia, neuritis, colds, influenza, lumbago, rheumatism, sleeplessness, menstrual pains, etc." *Formula:*—Calcium Acetyl Salicylate 94%, Calcium Chloride 5%, Sodium Chloride 1%. *Dose:* 1 teaspoonful.

California Syrup of Figs (*California Fig Syrup Co.: Proprietary Agencies Ltd., Successors, London*). *Formula:*—Contains approx. 27·8% Fluid. Ext. Senn. Fol. in Fig Syrup with flavouring essences. *Dose:* Adults, 1 or 2 tablespoonfuls; Children, $\frac{1}{2}$ to 2 teaspoonfuls.

Calsalettes Brand Tablets (*The Torbet Lactic Co., Edinburgh*). "Purely vegetable and lactic." *Formula:*—Ext. of Curacaoaloin 90·0, Amylum 39·0, Sacch. Lact. 7·33, Ac. Stear. 7·33. *Dose:* 1 to 4 tablets.

Calsaloids Brand Tablets (*The Torbet Lactic Co., Edinburgh*). "Purely vegetable and lactic. Uncoated." *Formula:*—Ext. of Curacaoaloin 90·0, Amylum 39·0, Sacch. Lact. 7·33, Hydrarg. Subchlor. 15·0, Ac. Stear. 7·33. *Dose:* 1 to 4 tablets.

Calsprate Brand Tablets (*Genatosan Ltd., Loughborough*). "Soluble Calcium Aspirin. Indications: Headache, nerve pains and rheumatic disorders, colds, influenza, chorea, menstrual pains, sleeplessness, fatigue, etc." *Formula:*—Calcium acetylsalicylas 77%, Excipient Lactose 23%. Each tablet weighs 8 gr. *Dose:* 2 tablets.

[P1] Calvert's No. 5 Carbolic Acid (*F. C. Calvert & Co. Ltd., Manchester*). Directions are given for disinfecting purposes, for use in the sick room, for subduing bees, and for use in farms and stables. *Formula:*—Contains 98/100% phenols w/w.

[P1] Calvert's No. 1 Carbolic Acid or Phenol (*F. C. Calvert & Co., Manchester*). A solution made by mixing 24 drops in an 8-ounce bottle of water (two tablespoonfuls of this solution contain a 3 gr. dose) will be found efficacious as a gargle or mouth wash.

[P2] Calvex Brand Ointment (*F. C. Calvert & Co. Ltd., Manchester*). "For burns, scalds, chilblains, chapped hands, sore heads or lips, chafed skin and other skin ailments. Sufferers from indolent ulcers, piles, carache, rheumatic and neuralgic pains and colds on the chest will derive much benefit by local application." *Formula:*—Cera Alb. Co. 26·2%, Phenol 8·1%, Camph. 4·4%, Cetac 8·8%, Ol. Oliv et Arach. 52·5%.

Carmarole Brand Compound (*International Laboratories Ltd., London*). "A most efficacious treatment for kidney and bladder troubles, rheumatism, general weakness and debility, dyspepsia, unnatural losses, anæmia, and for enriching and purifying the blood." *Formula:*—Potass. Acetate 5%, Ext. Barosma Betulina Liq. 11%, Ext. Arctostaphylos Uva-Ursi Liq. 12%, Ext. Agropyrum Repens. Liq. 16%, Oleum Menth. Pip. 0·05%, Oleum Juniper Bacc. 0·1%, Chloroform 0·2%, Ethyl Alcohol 7·5%, Glycerin to 100%. *Dose:* 8 to 15 drops.

Carmarole Brand Compound Tablets (*International Laboratories Ltd., London*). "A marvellous new tonic and general re-vitalizer for young and old; unsurpassed for people who have passed middle life. A most efficacious remedy for the treatment of kidney and bladder troubles, rheumatism, general weakness and debility, dyspepsia, unnatural losses, anæmia, and for enriching and purifying the blood." *Formula:*—Extract of Barosma Betulina $\frac{1}{4}$ grain, Extract of Bearberry leaves $\frac{1}{4}$ grain, Oleum Bacc. Juniper $\frac{1}{4}$ minim, Oil of Barosma Betulina $\frac{1}{4}$ minim, Potassii Nitras 1 grain, Resina Podophylli $\frac{1}{4}$ grain, Hexamethylenetetramine $\frac{1}{4}$ grain. *Dose:* 1 tablet.

Carovit Brand Chlorophyll Tablets (*Continental Laboratories Ltd., London*). "Contain chlorophyll and beta carotene. As both substances are present in the natural and unadulterated form in which they are found in certain green plants they exert their full synergistic action." *Formula:*—Carovit Chlorophyll 0·01 grm., Ferri Phosph. Solub. 0·008 grm., Calc. Phosph. 0·06 grm., Saccharose Ad. 0·125 grm. *Dose:* 1 to 3 tablets.

Carter's Brand Little Nerve Pills (*Carter Products, Inc., London, Thomas Manns Ltd., London*). "An effective nerve tonic for nervousness, headache, insomnia, stomach, indigestion, dyspepsia." *Formula:*—Aloin ·01 gr., Zinc phosphide ·005 gr., Podophylli ·01 gr., Ext. Damiane ·05 gr., Cascarin ·05 gr. *Dose:* 1 pill.

Carter's Little Liver Pills (*Carter Products, Inc., London, Thomas Manns Ltd., London*). "For liver disorders, constipation, indigestion, headache,

complexion, rheumatism." *Formula*:—Podophylli Resina 0.625, Aloe Curaco 0.25, Glycyrrhizæ 0.025, Acacia 0.06, Amylum 0.17, Excipients q.s. *Dose*: 1 to 4 pills.

Cassell's (Dr.) Brand Instant Relief (*Veno Drug Co. Ltd., Manchester*). "For liver troubles, sick headache, and constipation." *Formula*:—Phenolphthal. 10.884%, Jalap Res. 3.628%, Scammon Res. 3.628%, Podoph. Res. 0.907%, Sod. Phenolsulphon. 7.256%, Senn. Fol. 17.241%, Zingib. 12.698%, Sucrosum 31.745%, Acacia 6.349%, Ol. Menth. Pip. 0.112%, Oleores Zingib. 0.112%, Creta Gall. Pur. 5.440%. *Dose*: 1 tablet; Children, $\frac{1}{2}$ tablet.

Cassell's (Dr.) Tablets (*Veno Drug Co. Ltd., Manchester*). "Feed and nourish the nerves when impaired by nervous breakdown, anæmia, neurasthenia, insomnia, loss of appetite, indigestion and malnutrition." *Formula*:—Caffeina 4.89%, Magnesii Hypophosph. 0.99%, Mangani Hypophosph. 0.99%, Ferri Lactas 14.98%, Quinina Sulphas 0.50%, Kola 10.15%, Calcii Phosphas 19.75%, Cupri Sulphas 0.10%, Potassi Sulphas 2.00%, Calcii et Sodii Lactas 5.00%, Glucosum 32.87%, Papainum 2.00%, Ol. Petrolei Alb. 0.39%, Ol. Anisi 0.59%, Creta Gallica 4.80%. *Dose*: 2 tablets; young people, 1 tablet.

[P1] Castellian Brand No. 10 Cough Mixture (*John McGuffie & Co. Ltd., Liverpool*). "A famous remedy for bronchial catarrh, chronic bronchitis, coughs, colds, hoarseness, asthma, influenza." *Formula*:—Contains Morph. Acet. 0.036%, Antim. et Pot. Tart 0.045%, Ext. Glycyrrh. Liq. 1.00%, Ext. Scill. Liq. 1.50%, Acet. Scill. 2.70%, Ether 0.375%, Chlorof. 0.25%, Mucil. Acac. vel Lini 40.0%. *Dose*: 1 teaspoonful.

Castols (*Thomas Kerfoot & Co. Ltd., Bardsley*). "Nice as chocolate. No more Castor Oil." The composition of this preparation was not disclosed.

Castorets (*Carter & Sons, Sheffield*). "Tablets for constipation." Contain phenolphthalein 20%. *Dose*: 1 or 2 tablets.

Celmo Brand Tablets (*Celmo Ltd., London*). "For rheumatism, gout, rheumatic gout, sciatica, lumbago, eczema, neuritis, etc." *Formula*:—Potassium Iodide 1 gr., Ext. Gentian $\frac{1}{2}$ gr., Sodium Salicylate $\frac{1}{2}$ gr., Podophyllin $\frac{1}{8}$ gr., Aloin $\frac{1}{8}$ gr., Ext. Cascara Sagrada U.S.P. $\frac{1}{2}$ gr., Ext. Pichi $\frac{1}{2}$ gr., Oil Juniper $\frac{1}{2}$ min., Oil Peppermint $\frac{1}{2}$ min., Powd. Capsicum $\frac{1}{2}$ gr., Powd. Pepsin. 1:3000, $\frac{1}{2}$ gr., Powd. Jamaica Ginger $\frac{1}{2}$ gr., Quinine Sulphate $\frac{1}{30}$ gr., Powd. Charcoal $\frac{1}{2}$ gr., Excipients q.s. *Dose*: 2 tablets.

Celunox (*Celunox Ltd., London*). "The Celery treatment. Knocks out rheumatism. For rheumatism, gout, lumbago, sciatica and neuritis." *Formula*:—Ext. Celery Seed (Apium Graveolens) 0.016 g., Salicin 0.081 g., Phenacet. 0.065 g., Guaiac. Res. 0.016 g., Fraxinus Excelsior (Linneus) 0.002 g., Ext. Balm of Gilead 0.002 g., Cimicif. Res. 0.002 g., Gravel Root (Eupatorium Purpureum Linn.) 0.004 g., Aloin 0.001 g. *Dose*: 2 tablets.

Cephos (*Cephos Ltd., Blackburn*). "The physicians remedy. For neuralgia, headache and rheumatism." *Formula*:—Acetyl Salicylic Acid 573 parts, Acetyl Phenetidine 360 parts, Theine citrate 63 parts. *Dose*: 2 tablets.

Chameleon Oil (*Castle Laboratory, London*). Promotes circulation and is the most effectual penetrant known. For rheumatism, sciatica, lumbago, neuritis, neuralgia, toothache, headache, earache, backache, colds, sore throats, sprains and strains, stiff and swollen joints, chilblains, chapped hands, insect bites, etc." *Formula*:—Oil of Turpentine 28%, Witch Hazel Extract (distilled) 20%, Ox Gall. 14%, Water 14%, Liq. Ammonia 11%, Kerosene 7%, Spirit 4.2%, Camphor 0.7%, Essential Oils 2%.

Chardox Brand Super-Activated Charcoal (*E. Griffiths Hughes Ltd., Manchester*). "Immediate relief without drugs from indigestion, gas, acidity, flatulence, heartburn, stomach pains, gastritis, colitis and all gastric and intestinal disorders."

Chardox Tablets (*E. Griffiths Hughes Ltd., Manchester*). "Highly activated charcoal. Adsorbs and fixes toxins, gases and products of faulty digestion, both gastric and intestinal. It is thus indicated in cases of intestinal toxæmia, gastric fermentation, halitosis, intestinal auto-intoxication, colitis, diarrhæa, and other inflammatory and infective conditions of the gastro-intestinal tract." *Formula*:—Each tablet contains 2.925 grs. of Chardox, a highly-activated pure vegetable charcoal (Carbo Activatus). *Dose*: 1 to 3 tablets.

Chilblain Ointment (*Sanarol Brand Products, London*). "P.J.F. 0266. Quickly reduces inflammation and allays irritation." *Formula*:—Adeps Lanæ 20 parts, Paraff. Moll. Flav. 74 parts, Paraff. Dur. Alb. 10 parts, Camphor 8 parts, Phenol 1 part, Ol. Lavand. 1 part, Ol. Rosemary 2 parts, Bals Peru 2 parts. **[P2] Chililine** (*Osborne, Bauer & Cheeseman Ltd., London*). "A remedy for chilblains. Gives immediate relief. A few applications will effect a cure."

Formula:—Active constituents: Phenol 5%, Glycerin 10%, Paraff. Moll. Flav. 10%.

[P1] **Chocoloids** (*Squire & Co., Birmingham Ltd., London*). "A scientific remedy prepared by British chemists and intended as a cure for constipation and the maladies arising from this condition of the intestines, such as hæmorrhoids, congestion of the liver, headache, want of appetite, etc."

Formula:—Ext. Coloc. Co. 38.08%, Ext. Jalapæ 19.04%, Podophyllin 4.76%, Pulv. Leptandrin 9.52%, Ext. Hyoscy. Sicc. B.P. 9.52% w/w, Ext. Tarax. 9.52%, Pulv. Capsici 9.52%. **Dose:** 1 or 2 tablets.

Cicfa Tablets (*Cicfa Co. Ltd., Watford*). "For both stomach and intestinal indigestion. Cure indigestion and thus prevent constipation, flatulence, acidity, heartburn. Ensures regular elimination of waste products, thus preventing rheumatism, lumbago, sciatica, gout and similar conditions."

Formula:—Carbo Ligni Purificatus 18.09%, Pepsinum 48.24%, Diastaseum 16.08%, Aloinum 1.51%, Ext. Casc. Sagr. Sicc. 16.08%. **Dose:** 1 or 2 tablets.

Clark's Brand Glycola Foot Cream (*The manufacturer's address is not given on the container*). "Specially prepared for those whose feet are liable to tiredness, soreness or chilblains."

Formula:—Camphor 0.323%, Tinct. Benzoin. 16.796%, Ol. Lavand. 0.258%, Vegetable Oil 82.623%.

Clarkes Brand Blood Mixture (*The Lincoln and Midland Counties Drug Co. Ltd., Lincoln*). "It is highly recommended for blood and skin complaints, eczema, psoriasis, eruptions, sores, ulcers, ulcerated legs, enlarged glands, rheumatism, gout, etc."

Formula:—Potass. Iod. 1.084%, Sod. Salicyl. 1.304%, Sod. Nuclein. 0.200%, Potass. Bicarb. 0.865%, Ammon. Chlorid. 0.652%, Liq. Gent. Co. C. 0.107%, Trichloromethane 0.237%, Spt. Vini Rect. 0.237%, Sol. Colour 0.498%, Aqua Dest. 94.816%. **Dose:** 1 teaspoonful to 1 tablespoonful.

Clarkes Brand Blood Mixture (Tablet form) (*The Lincoln and Midland Counties Drug Co. Ltd., Lincoln*). "Highly recommended for blood and skin diseases, rheumatism, gout, eczema, psoriasis, boils, arthritis, muscular rheumatism, lumbago, eruptions, sores, ulcers, ulcerated legs, etc."

Formula:—Pot. Iod. 20.492%, Sod. Salicyl. 24.590%, Pot. Bicarb. 16.393%, Am. Chlor. 8.197%, Ext. Gent. Sicc. 4.099%, Ol. Cassiæ 1.639%, Ext. Saccharom Cerevis 24.590%. **Dose:** 2 tablets; Children, $\frac{1}{2}$ to 1 tablet.

Clarkes Brand Salve (*The Lincoln and Midland Counties Drug Co. Ltd., Lincoln*). "The best application for skin complaints, eczema, ulcers, ulcerated legs, sores, etc."

Formula:—Zinci Oxid. 10.000%, Ac. Hydroxybenzoic 1.5%, Thymol 0.25%, Resinæ Coloph. Purif. 10.000%, Paraff. Dur. 3.50%, Paraff. Moll. Flav. 74.75%.

Clarkes Brand Skin Lotion (*The Lincoln and Midland Counties Drug Co. Ltd., Lincoln*). "Cleansing, antiseptic, soothing and healing."

Formula:—Liq. Carb. Det. 10.00%, Sodii Biorat. 2.50%, Ol. Thym. Vulg. 0.05%, Glycerol 11.50%, Aqua Dest. 85.95%.

Clarkson Brand Medicine (*Clarkson, Ryde, I.O.W.*). "For internal and external use. For dysentery and all internal pains and for influenza, toothache, ear-ache, etc."

Formula:—Tr. Zingib. 1.250, Tr. Myrrhæ 1.875, Spt. Camphor. 1.875, Pulv. Capsici 7.000, Pulv. Cocci Cacti 0.062, Spt. Vini Rect. 50.000, Aqua ad. 100.000. **Dose:** Adults, 10 to 40 drops.

Clynol Brand Berries (*Proprietors: Dearborn (1923) Ltd., London*). "For obesity."

Formula:—Each capsule is guaranteed to contain Ext. Fucus Vesiculosus Gr. 2, Phenolphthalein Gr. $\frac{1}{4}$, Excipient q.s. **Dose:** 1 capsule.

Cockles Brand Antibilious Pills (*James Cockle & Co. Ltd., London*). "Purely vegetable in composition, perfectly harmless and quite gentle in action."

Formula:—Aloes Soc. 17.72%, Aloes Barb. 17.72%, Colocynth 8.86%, Pulv. Anthem. Flor. 8.86%, Solazzi 8.86%, Gamboge 8.86%, Excipient 20.26%. **Dose:** 1 to 4 pills.

Coles' Family Ointment (*The Coles Syndicate, Reading*). "Cures inflamed sore throats, bad legs, white swellings, abscesses, boils, poisoned fingers, etc."

Formula:—Burgundy Pitch 3%, Galbanum Plaster 17%, Honey 9%, Japan Wax 14%, Cobaiba Balsam 4%, Venice Turps. 4%, Spermaceti 12%, Olive Oil 23%, Vaseline 14%.

[P1] **Collis Browne's (Dr.) Chlorodyne** (*J. T. Davenport, London*). "The medical properties of this remedy are anodyne, diaphoretic, sedative, astringent, antispasmodic. As a remedial agent in the treatment of febrile, inflammatory or neuralgic affections its administration has been found to exercise most remarkable curative effects in all stages of the disease."

Formula:—Ext. Opii Liq. (10% morphine) 1.4% w/v, Codeine .21% w/v, Chloroform 14% v/v,

Proof Spirit 5.75% *v/v*, Tinct. Capsicine Rect. .31% *v/v*, Ol. Menth. Pip. .05% *v/v*. *Dose*: 10 to 30 drops in a wineglassful of water.

Compericum (*W. G. Heath, Leamington Spa*). "An invaluable application for abrasions of the skin from whatever cause arising, and in all cases where it is broken or inflamed." *Formula*:—A.C. Tannic 0.05%, Anchusa *q.s.*, Flor. Hypericum 6%, Ol. Arachis 93%.

Compound Balsam of Aniseed (*Keybells, Derby*). "Contains Compound Essence of Linseed, Malt and Liquorice, Potass. Nitrate, Extracts of Horehound, Aniseed and Ipecacuanha, etc." *Dose*: Adults, 1 or 2 teaspoonfuls; Children, $\frac{1}{2}$ to 1 teaspoonful.

Condy's Crystals (*Distributors: Savory & Moore Ltd., London*). "Non-poisonous germicide." *Formula*:—Contains 7% to 8% of the sodium compounds of manganic and permanganic acids with sodium chloride and sodium nitrate.

Condy's Fluid Germicide (*Distributors: Savory & Moore Ltd., London*). "Nature's great purifier and cleanser. Valuable for personal hygiene, domestic and sanitary use. A safe antiseptic and deodorant." *Formula*:—Contains twice its volume of available oxygen derived from the 1% of the sodium compounds of manganic and permanganic acid contained by this fluid, also sodium nitrate and 3 to 4% of sodium chloride.

Condy's Remedial Fluid (*Distributors: Savory & Moore Ltd., London*). "The oxygen treatment. The sovereign cure for sore throats, wounds, sores, discharges (injection), confinements, sore eyes, indigestion, diarrhoea, ulcers, burns, etc." *Formula*:—Contains twice its volume of available oxygen derived from 1% of the sodium compounds of manganic and permanganic acids, sodium nitrate, and 0.6% of a sodium compound of boric acid.

Congreve's Elixir (*G. T. Congreve Ltd., Horsham*). "It has given relief in chronic bronchitis, asthma, emphysema, obstinate cough, colds, hoarseness, difficult breathing and the effects of catarrhal influenza." *Formula*:—Bals. Tolu 2%, Tereb. Venet. Fact. 2%, Catech. 2%, Guaiac. Res. 1%, Cocc. 0.25%, Scill. 0.5%, Marrub. 0.5%, Tussilag. Fol. 0.5%, Hyssop. Offic. 0.25%, Rosmarin. Offic. 0.375%, Sp. Tenuior. 51%, Aq. ad 100%. *Dose*: For adults, 1 teaspoonful.

Constipon Brand Laxatives (*Constipon Ltd., Glasgow*). "Nature's own laxative." *Formula*:—Each tablet contains Chocolate $1\frac{1}{2}$ grains, Phenolphthalein $1\frac{1}{2}$ grains, Saccharum $6\frac{1}{2}$ grains, G. Acacia $\frac{1}{4}$ grain. *Dose*: 1 to 2 tablets; Children, $\frac{1}{2}$ tablet.

Curicones (*Stephen Matthews & Co. Ltd., London*). "For all cases of rheumatism, lumbago, rheumatoid arthritis, neuritis, sciatica, gout, etc." *Formula*:—Sulphur 19.7, Sodii Bicarbonas 5.6, Cimicifuga 3.5, Guaiaci Resina 2.1, Acid Acetylsalicyl 3.5, Sodii Benzoas 1.4, Caseinum 3.5, Zingiber 0.7, Excipient ad 100. *Dose*: 1 or 2 capsules.

Cuticura Brand Ointment (*Made in England, as prepared by Cuticura Laboratories, Malden, Mass.*). "A super-creamy emollient. For irritations of the skin and scalp." *Formula*:—Fluid Mineral Oil 36.38% (Sulphur .35%), Semi-Solid Petroleum 33.76% (Sulphur .048%), Solid Mineral Oil Wax 26.35%, Bees Wax Natural 2.55%, Pine Oil .04%, Rose Geranium .17%, Chlorophyll .04%, Precipitated Sulphur B.P. .50%, Phenol B.P. .16%.

[P1-S1] Cwander Brand Coryzine (*A. Wander Ltd., London*). "A preparation for application inside the nostrils in nasal catarrh, hay fever, etc." *Formula*:—Menthol 5.34, Acid Boric. 26.75, Cocaine Hydrochlor. 0.188 *w/w*, Thymol 0.084, Ol. Lavand. 0.94, Paraffin Molle 241.0.

Cwander Brand Hemorrhoidal Salve (*A. Wander Ltd., London*). *Formula*:—Resorcin 1.1 part, Bismuth Oxy-iodo-gallate 9 parts, Zinc Oxide 12 parts, Paraffin Dur. 6 parts, Paraffin Molle Flav. 118 parts.

Cystex Brand (*Knox Labs. Ltd., London*). "Kidney, bladder and rheumatism treatment." *Formula*:—*Grey Tablets*: Hexamethylenamine 2 $\frac{1}{2}$, Salol $\frac{1}{2}$, Benzoic acid $\frac{1}{2}$ (Grns.); *Brown Tablets*: Extract Buchu $\frac{1}{2}$, Extract Corn silk $\frac{1}{2}$, Extract Triticum $\frac{1}{2}$, Potassium Bicarbonate 1, Acetphenetidin 1, Sodium Borate $\frac{1}{2}$ (Grns.).

Cystopurin (*Genatosan Ltd., Loughborough*). "Safe internal antiseptic. Combats all blood borne infections. For kidney and bladder infections." *Formula*:—Hexamethylenetetramine 33.98%, Sodium Acetate (Anhydrous) 39.83%, Water of Crystallisation 26.19%. In chemical combination. Each tablet weighs 1 g. *Dose*: 1 to 3 tablets.

Daisy Brand Tablets (*J. E. Ellis Ltd., Leeds*). "For headache, neuralgia, tic, toothache and all nerve pains, rheumatism, neuritis, lumbago, sciatica, periodical pains, influenza, colds, etc." *Formula*:—Caffeine 1.67, Piperyl-

piperidine .17, Acetphenetidin 24.98, Acid. Acetylsalicyl. 54.16, Amylum et excipients 19.02. *Dose:* 2 tablets.

✓ **Daisy Powders** (*J. E. Ellis Ltd., Leeds*). "Cures in a few minutes headache, neuralgia and all nerve pains. Speedily relieves rheumatism, lumbago, sciatica. A specific for colds, chills, influenza." *Formula:*—Acetphenetidin 44.44, Ac. Acetylsalicyl. 55.56. *Dose:* 1 powder.

✓ **Damaroids** (*The Hygienic Stores, London*). "The Key to Youth. For general weakness, convalescence, nervous debility, chronic exhaustion, etc." *Formula:*—Aloin gr. $\frac{1}{16}$, Orchitic sub. gr. $\frac{1}{2}$, Ext. Damiana gr. $\frac{1}{2}$, Hexamin. gr. 1, Ferri Hypophosph. gr. $\frac{1}{2}$, Glycyrrh. q.s., Sucrose q.s. in each tablet. *Dose:* 1 or 2 tablets.

✓ **Davis's (Dr.) Famous Female Pills** (*Dr. Davis's Female Pills, London*). "Are world-renowned and unequalled. An excellent and effective remedy for anæmia, giddiness, fulness and swelling after meals, loss of appetite, palpitation of the heart, depression, weakness, irregularity and all female ailments." *Formula:*—Sulph. Iron .68, Rue Pulv. .89, Cape Aloes .66, Colocynth. .22, Gum Acacia .89, Cayenne .12, Myrrh. .89, Ginger .22, Tansy .44. *Dose:* 2 pills.

D.D.D. Prescription (Extra Strong) (*D.D.D. Company, London*). "For eczema and all diseases of the skin and scalp." *Formula:*—Chlorbutol 1.11%, Resorcinol .75%, Methyl Salicylate .95%, Phenol .98%, Thymol .09%, Menthol .15%, Salicylic Acid 2.22%, Spirit 34.28%, Aqua ad 100% by weight.

D.D.D. Prescription (Ordinary strength) (*D.D.D. Company, London*). "The remedy for eczema, dermatitis, bad leg, ringworm, cuts and wounds, burns and scalds, itching piles, psoriasis." *Formula:*—Chlorbutol 1.11%, Resorcinol .75%, Methyl Salicylate .81%, Phenol .98%, Thymol .09%, Menthol .15%, Salicylic Acid .74%, Spirit 33.90%, Aqua ad 100% by weight.

Deakin's Inflammation Remedy (*G. Deakin and Hughes Ltd., Failand, Nr. Bristol*). "For pains, chills, fevers, influenza, inflammations, ulcers, pleurisy and liver, stomach and kidney diseases." *Formula:*—Glycerin 0.50%, Inf. Buchu. Conc. 1.00%, Pinus Canadensis 0.15%, Marrubium Vulgare 0.15%, Chloroform 0.50%, Eupatorium Perfoliatum 0.15%, Asclepias Tuberosa 0.15%, Liq. Ext. Gentiana Lutea 0.07%, Phenol Liq. 0.41%, Liq. Ext. Cimicifugæ 0.20%, Ol. Menth. Pip. 0.04%, Liq. Ext. Guaiacum 0.10%, Liq. Ext. Sambucus Nigra 0.15%, Liq. Ext. Arctium Majus 0.50%, Camphor 0.04%, Liq. Ext. Parietaria 0.15%, Ol. Cinnamon 0.05%, Liq. Ext. Galium Aparine 0.20%, Sodium Citras 3.00%, Liq. Ext. Juniper 0.20%, Sodium Salicylas 3.41%, Liq. Ext. Uva Ursi 0.20%, Myrica Cerifera 0.30%, Color q.s., Aqua ad 100%.

Deakin's Lung Healer (*G. Deakin and Hughes Ltd., Failand, Nr. Bristol*). "For coughs, colds, tight breath, asthma, whooping cough, bronchitis and shortness of breath." *Formula:*—Dec. Cetraria Islandica .220%, Ol. Cinnamon .030%, Liq. Ext. Sticta Pulmonaria .143%, Syr. Lobelia 1.100%, Liq. Ext. Asclepias Tuberosa .110%, Aq. Chlorof. Conc. .022%, Liq. Ext. Hyssop. .110%, Tinct. Pruni Serot. .121%, Liq. Ext. Symphytum .110%, Sucrose 22.532% (or Sacchari Faex 33.800%), Liq. Ext. Glycyrrhizæ .022%, Ol. Menth. Pip. .015%, Camphor .060%, Phenol Liq. .375%, Chloroform .900%, Acet. Scillæ .022%, Tinct. Ipecac. 8.300%, Tinct. Senegæ .027%, Color q.s., Aqua add 100%. *Dose:* Adults, 1 or 2 teaspoonfuls.

Dettol Antiseptic (*Reckitt & Sons, Hull*). "Dettol is a germicide three times more efficient (Rideal-Walker test) than pure Carbolic Acid, yet it is non-poisonous and non-staining." *Formula:*—Active constituents: Chloroxylenol 3%, Terpeneol 12%.

Dettol Brand Ointment (*Reckitt & Sons, Hull*). "For the treatment of boils, whitlows, carbuncles, etc., impetigo, 'Barber's Rash,' herpes (cold spots), ulcers, chapped hands, burns and scalds, etc." *Formula:*—Adeps Lanæ 36.68% w/w, Cera Lanæ 4.58% w/w, Paraff. Molle Alb. 45.90% w/w, P. chlor. m. xyleneol 0.23% w/w, Acid Salicyl 2.75% w/w, Menthol 0.23% w/w, Ol. Eglanter. Synth. 0.46% w/w, Aqua 9.17% w/w.

Dettol (Instrument) (*Reckitt & Sons, Hull*). "For sterilising surgical instruments. Forms a clear solution with soft or distilled water. 2½ times more efficient than pure carbolic acid (Rideal-Walker test)." *Formula:*—Active constituents: Chloroxylenol 3%, Terpeneol 12%.

Dettol Obstetric Cream (*Reckitt & Sons, Hull*). *Formula:*—Active constituents: Chloroxylenol 1.4% w/v, Terpeneol 1.8%.

Dettolin Brand Gargle (*Reckitt & Sons, Hull*). "Dettolin is germicidally as efficient as a 50% solution of pure Carbolic acid (Rideal-Walker test)." *Formula:*—Dimethyl Chlor Phenyl Hydrate 1.02% Menthol 0.12%, Ol.

menth. pip. 0.50%, Ol. anis. 0.10%, Sapo vegetalis 0.50%, Glusidum 0.30%, Pot. carb. 1.00%, Sp. meth. ind. 65.00%, Chlorazol pink 0.0045%, Aq. dest. ad 100 vols.

De Witt's Antacid Powder (*E. C. De Witt & Co., London*). "Guaranteed for indigestion and all stomachic affections." *Formula*:—Mag. Carb. 15.54%, Calc. Carb. 24.28%, Sodium Bicarb. 48.18%, Coll. Kaolin 8.74%, Bismuth. Subgall 1.00%, Bismuth Subcarb. 1.00%, Malt. Diastase 1.00%. *Dose*: 1 teaspoonful in water or milk.

De Witt's Brand Kidney and Bladder Pills (*E. C. De Witt & Co., London*). "For weak kidneys, rheumatism, inflammation of the bladder, backache, scalding or scanty urine, too frequent desire to urinate, gravel and all uric acid complaints." *Formula*:—Pichi, 7.17%, Ext. Casc. Sag. 10.52%, Meth. Blue 2.39%, Pot. Nit. 25.82%, Ext. Uva Ursi 10.52%, Buchu Fol. 2.87%, Podoph. 54%, Excip. q.s. *Dose*: 1 or 2 pills.

De Witt's Brand Little Laxative Pills (*E. C. De Witt & Co., London*). "For liver complaints, constipation, biliousness, headache, indigestion, dizziness, dyspepsia, the complexion, etc." *Formula*:—Aloin 31.25%, Capsicine 3.13%, Podophyllin Emodi 15.62%, Gingerine 3.13%, Cascara 3.13%, Excip. q.s. *Dose*: 1 or 2 pills.

[P1] Dacey's Dr. Bateman's Drops (*Sutton & Co., London*). "An ideal remedy for colds and influenza." *Formula*:—Pulv. Castoreum .052, Camphor .416, Oil Anisi .208, S.V.R. 58 o/p 12.5, Morphine Anhydrous .084, Sacch. Ust. 1.04, Pot. Carb. .208, Aqua Ad. 100.0. *Dose*: Adults, 1 teaspoonful; Children, 5 to 15 years, $\frac{1}{2}$ teaspoonful.

Digestif Rennie's Tablets (*E. Griffiths Hughes Ltd., Manchester*). "The ideal remedy for indigestion, dyspepsia, flatulence, heartburn, biliousness, acidity, etc." *Formula*:—Bismuthi Carbonas 0.0131 gms., Magnesii Carbonas 0.0698 gms., Pepsinum 0.0011 gms., Pancreatinum 0.0007 gms., Bismuthi Oxidum 0.0015 gms., Magnesii Hydroxidum 0.0015 gms., Magnesii Oxidum 0.0029 gms., Kaolinum Colloidalis 0.0029 gms., Calcii Phosphas 0.0003 gms., Lactosum 0.024 gms., Magnesii Stearas 0.003 gms., Oleum Menthae Piperitæ 0.0025 gms., Calcii Carbonas 0.8486 gms., Sacrosum 0.2144 gms., Acaciæ Gummi 0.061 g.

Dimol A Brand (*Dimol Laboratories, London*). "For intestinal disinfection." *Formula*:—Dimol (Dimethylmethoxyphenol) 20%, Pulv. Glycyrrhizæ 40%, Sacc. Alb. 16.25%, P. Amylum 10%, P. Dextrine 13.75%. 5 grains in each tablet. *Dose*: 1 to 3 tablets.

Dimol B (*Dimol Laboratories Ltd., London*). "For stomachic and intestinal disinfection." *Formula*:—Dimol (Dimethylmethoxyphenol) 20%, Pulv. Glycyrrhizæ 40%, Sacc. alb. 16.25%, P. Amylum 10%, P. Dextrine 13.75%. 5 gr. in each tablet. *Dose*: 1 to 3 tablets, or as ordered by the physician.

Dinneford's Magnesia Tablets (*Dinneford & Co., Brentford*). "Antacid—Aids digestion." *Formula*:—Each tablet contains approximately 5.33 grains of pure Magnesium Hydroxide. *Dose*: 1 or 2 tablets.

Dinneford's Pure Fluid Magnesia (*Dinneford & Co. Ltd., Brentford*). "Is an agreeable and effective means of neutralising excessive stomach acidity, thereby affording prompt alleviation of all the digestive disturbances which arise from it." *Formula*:—Liquor Magnesii Bicarbonatis. *Dose*: 1 or 2 teaspoonfuls.

Diotex Brand Tablets (*Knox Laboratories Ltd., London*). "For stomach troubles." *Formula*:—Magnesium Oxide Lt. 2 Grs., Pepsin Po. Insol. 1.3000 $\frac{1}{10}$ Gr., Pancreatin $\frac{1}{2}$ Gr., Duodenal Sub. Po. Desic. $\frac{1}{2}$ Gr., Oil of Cinnamon $\frac{1}{10}$ Gr., Saccharin Soluble $\frac{1}{2}$ Gr., Charcoal q.s., Excipient q.s. *Dose*: 2 tablets.

Dismenol Brand Tablets (*Roberts & Co., London*). "Allay the spasmodic and continuous dull pains of menstruation." *Formula*:—Each tablet contains Paraulphamidobenzoic Acid 0.05 gm., Antipyrin 0.25 gm., Lactose 0.25 gm. *Dose*: 1 tablet.

Diuramil Brand (*Pharmax Ltd., London*). "Uric acid solvent. For acute attacks of rheumatism, gout, sciatica, urinary lithiasis, gravel, stone, renal colic, cystitis, arthritis, and other similar ailments." *Formula*:—Pip. Tart. 3.321, Pip. Cit. 2.223, Hexamine 3.500, Lith. Sal. 0.800, Lith. Benz. 0.725, Disoda. Phos. 2.325, Effervescent base q.s. ad 100. *Dose*: 1 teaspoonful.

Doan's Backache and Kidney Pills (*Foster McClellan Co., London*). "Diuretic urinary antiseptic and stimulant." *Formula*:—Ext. Uva Ursi 25.000%, Ext. Buchu 3.125%, Ext. Gent. 9.375%, Aloin 0.391%, Potass. Nitrat. 16.667%, Ol. Junip. Bacc. 1.333%, P. Fol. Buchu 4.687%, P. Fol. Uva Ursi 10.417%, Mag. Oxid. 6.250%, Excip. q.s. *Dose*: 1 to 3 pills.

Doan's Ointment (*Foster McClellan & Co., London*). "For piles, eczema and itching skin complaints." *Formula*:—P. Zinc. Ox. Puriss. 13.33%, Hydrarg. Subchlor. 17.78%, Phenol 1.67%, Paraff. Dur. 5.56%, Paraff. Moll. Fl. ad 100.00%.

Dodds' Brand Kidney Pills (*The Dodds Medicine Co. Ltd., Consignees: Merchandising and Servicing Corporation Ltd., London*). "For rheumatism, tired feeling, urinary troubles. For female weakness, purifies the blood, cleanses the system. For backache, headache, bladder troubles." *Formula*:—Ext. Buchu .00324 gm., Ext. Uva Ursi .00324 gm., Ol. Juniperis .0162 gm., Sod. Bicarb. .0648 gm., Pot. Nit. .648 gm., Saponis .0324 gm., Pod. Res. .00178 gm., Excip. q.s. *Dose*: 1 to 3 pills.

[P1] **Do-Do Brand Tablets** (*International Laboratories Ltd., London*). "Banish asthma. They are of the utmost value in the treatment of bronchial asthma, bronchitis, night coughing, fits, chest wheeziness, dyspnoea (difficult breathing), emphysema (air distended lungs)." *Formula*:—Ephedrine Hydrochloride $\frac{1}{2}$ grain, Grindelia Powder $\frac{1}{2}$ grain, Acetylmethyldimethylxamidophenylhydrazine (Pickups Ltd.) $\frac{1}{2}$ grain, Theobromine Cal. Sal. $\frac{1}{2}$ grain, Lithium Benzoate 1 grain. *Dose*: 1 tablet.

Do-Do Pastilles (*International Laboratories Ltd., London*). "Prophylactic against colds, catarrh, etc." *Formula*:—Ext. Fucus Vesie (Fucaceæ) 7.5%, Mucilago Acacia 45%, Ext. Rubi Nig. 36.5%, Acidum Tartaricum 1%, Saccharum Album 10%.

Dutch Drops (*Tilly*). "Used for generations in cases of kidney and bladder disorders, stomach and liver troubles, pains in the back, skin eruption, painful menstruation, etc., sore gums, ulcers and bruises." *Formula*:—Spiritus Terebinthinæ 10.40, Oleum Lini 9.40, Sulphur Sublimatum 1.00.

Eade's Antibilious Pills (*George Eade Ltd., London*). "One of the best medicines for bilious and liver complaints, disorders of the stomach and bowels, indigestion, loss of appetite, flatulency, heartburn, pains and giddiness of the head, costiveness, eruptions of the skin, etc." *Formula*:—Aloe 32.0, Scammon. Res. 16.0, Colocynth. 8.0, Zingib. 4.0, Sapo Dur. 4.0, Lactos 8.0, Ext. Gent. 12.0, Pil. Hydrarg. 16.0. *Dose*: 1 or 2 pills.

[P1] **Eade's Rheumatic and Gout Pills** (*Eade's Pills Ltd., London*). "Eade's Pills free you from the torturing pains of all rheumatic afflictions." *Formula*:—Ext. Colch. Sicc. 25.0, Aloe 12.5, Acac. 5.5, Guaiac. Res. 1.38, Lactos 2.76, Colch. Corm. 32.0, Glycyrrh. 16.0, Excip. 4.86. *Dose*: 1 pill.

Eade's Universal Anodyne (*George Eade Ltd., London*). "For the relief of pain, excellent for sprains, bruises, rheumatism, lumbago, sciatica, neuritis and in place of mustard plaster for colds, congestion, bronchitis." *Formula*:—Paraff. Moll. Flav. 72.49%, Paraff. Dur. 10.36%, Ol. Camph. Rect. 9.06%, Methyl. Salicyl. 4.53%, Menthol 2.26%, Oleores. Capsic. 1.30%.

Ecsolent Compound (*Ecsolent Buildings, London*). "For the treatment of eczema and skin troubles. Soothing and healing. Allays all irritation." *Formula*:—Paraff. Dur. 6, Zinci Oxidum 10, Amylum 10, Ac. Boric. 3, Ac. Salicyl. 2, Ol. Betulæ Alb. 0.5, Bals. Peruvian 0.2, Ol. Pini Conif. 0.2, Liq. Picis Alkali. 0.3, Paraff. Moll. Alb. to 100.

Egyptian Salve (*Reade Bros. & Co., Wolverhampton*). "For sores, wounds, ulcers, abscesses, burns, scalds, bad legs, inflamed eyes, chilblains, chapped hands, roughness of the skin, eruptions and skin diseases." *Formula*:—Camph. 0.54, Zinc. Oxid. 3.8, Acid. Boric. 1.6, Borax 0.8, Phenol Liq. 0.13, Ol. Arach. 21, Adeps Lan. Hydros. 9.1, Cera Alb. 5.7, Paraff. Moll. Alb., etc., ad 100%.

Elasto Brand Nature Salve (*New Era Treatment Co. Ltd., London*). "External treatment for ulcers, eczema, wounds, inflamed patches, sore joints, rheumatism, varicose veins, chilblains, sore and tender feet, eruptions of all kinds, skin diseases, piles, boils, bruises, sprains, burns, chapped lips and hands, sore breasts, etc." *Ingredients B.H.P.*: Calcii Phosphas. 6x 1.25%, Potassii Phosphas. 3x 1.25%, Magnesii Phos. 6x 1.25%, Calcii Fluoridum 6x 1.25%, Ointment Base to 100.

Elasto Brand Tablets (*New Era Treatment Co. Ltd., London*). "Elasto is the natural remedy for debilitated blood and all ailments arising therefrom. It is not a drug, but a vital cell-food which must be present in the blood to ensure complete health. For varicose veins, varicose eczema, bad leg, piles, poor circulation, heart troubles and all relaxed conditions." *Ingredients B.H.P.*: Calcii Phosphas 6x 25%, Potassii Phosphas 3x 25%, Magnesii Phosphas 6x 25%, Calcii Fluoridum 6x 25%. *Dose*: 2 tablets.

Elliman Athletic Rub (*Elliman, Sons & Co. Ltd., Slough*). "Refreshes and re-invigorates the muscles. Promotes liveness, agility, endurance and physical strength." The composition of this preparation was not disclosed.

Elliman's Universal Embrocation (*Elliman, Sons & Co. Ltd., Slough*). "For rheumatism and lumbago, sore throat from cold, cold at the chest, strains and mild sprains, training." *Formula*:—Active constituents: Ol. Terebinthinae essentia 35.41%, Acidum aceticum 4.16%.

Elmbalm Skin Ointment (*E. H. Beckett, Knot End-on-Sea*). "For bad legs, boils, abscesses, eczema, septic sores, and all skin diseases. Draws out the poison—then heals." *Formula*:—Pulv. Ulmi Fulv. 12.25%, Ol. Olivæ 12.25%, Ac. Boric. Pulv. 1.6%, Benzoinum 1.5%, Adeps. Prep. 22.0%, Petrolatum 22.0%, Resorcinum 2.5%, Zinci Oxid. Pur. 4.0%, Cholesterol Pur. 22.0%.

Endrine Brand Nasal Compound (*John Wyeth & Brother Ltd., London*). "A soothing antiseptic compound for the prompt relief of acute coryza (head colds), catarrh, sinusitis, asthma, bronchitis and hay fever." *Formula*:—Ephedrine 0.75%, Menthol 0.5%, Camphor 0.5%, Eucalyptol 0.5%, Oleum Ricini 0.5%, Liquid Paraffin q.s. 100%.

Eno's Fruit Salt (*J. C. Eno Ltd., London*). "The unrivalled health drink of young and old." *Formula*:—Contains approximately: Sod. Bicarb. 54.25%, Acid. Tart. 37.50%, Acid. Cit. 6.25%, Sod. Tart. 1.00%, Sod. Cit. 1.00%.

E.N.T. Capsules (*E.N.T. Manufacturing Co. Ltd., Bristol*). "For headache, toothache and neuralgia. For influenza, rheumatism, etc." *Formula*:—Each capsule contains 6 gr.—Acid Acetylsalicylic 7.4%, Antifebrin 28.6% w/w. *Dose*: 1 or 2 capsules.

E.N.T. Nerve Powders (*E.N.T. Manufacturing Co. Ltd., Bristol*). "Ends neuralgia troubles. Cures the most violent headache." *Formula*:—Acetyl salicylic acid add to 100. Antifebrin 0.83%. *Dose*: 1 powder; Children, $\frac{1}{2}$ powder.

[P1] **Ephazone** (*The Ephazone Co., London*). "A specialist's infallible prescription that ends asthma, bronchitis, bronchial asthma, emphysema, hay fever." *Formula*:—Ephed. Hydrochlor. 4.96% w/w, Theophyll. 7.86%, Iodo-theophyll.-calc.-salicyl. 4.96%, Phenazon. 19.84%, Calc. Benzylphthalas 2.87%, Fluoresc. Solub. 5.2%, Calc. Glucon. 4.8%. *Dose*: 1 or 2 tablets; Children, $\frac{1}{2}$ to $\frac{1}{4}$ tablet.

Ephedan Brand (*Clay & Abraham Ltd., Liverpool*). "Prescribed daily by the most eminent specialists and medical men for asthma, bronchitis, catarrh. Immediate relief for breathlessness, tightness of chest and other pulmonary trouble." *Formula*:—Calc. Gluconate 1.375; Ephedrine Hydrochlor. .950; Theobromine 2.0; Acid Acetyl Salicyl 4.0; Flavouring q.s.; Colouring q.s., Sacch. Lact. to 100. *Dose*: 1 or 2 powders; Children, $\frac{1}{2}$ to $\frac{1}{4}$ powder.

Ephedrol Brand (*Clay & Abraham Ltd., Liverpool*). "Gives instant relief in catarrh, hay fever, colds. A scientific method of application." *Formula*:—Ephedrine .95, Menthol 2.0, Camphor 2.0, Alkannin .05, Aromatic Oil .166, Paraffin Liq. Leve ad 100.

[P1-81] **Ergoapiol** (*Smith*) (*Distributors: Thos. Christy & Co. Ltd., Farnham*). "This preparation is designed expressly for physician's use under whose advice it is to be administered." *Formula*:—Each capsule contains: Ergot Præparata B.P. 1 gr., Oil of Pennyroyal $\frac{1}{2}$ min., Apiole U.S.P. IX (green) 5 min., Aloin B.P. $\frac{1}{2}$ gr., Castor Oil q.s. *Dose*: 1 or 2 capsules as prescribed by physician.

Estersil Brand (*Johnson & Sons*). "Recommended by the medical profession for the treatment of rheumatic affections, lumbago, sciatica, neuritis, stiff joints, etc." *Active constituents* 50% approx. each:—Ethyl Ester Salicyl-Glycollic Acid, Propyl Ester Salicyl-Glycollic Acid. $\text{OH}\cdot\text{C}_6\text{H}_4\cdot\text{CO}\cdot\text{OCH}_2\text{COOC}_2\text{H}_5$, 49.75%, $\text{OH}\cdot\text{C}_6\text{H}_4\cdot\text{CO}\cdot\text{OCH}_2\text{COOC}_2\text{H}_5$, 49.75%, Ol. Lavand. 0.50%.

Evans's Antiseptic Throat Pastilles (*Evans, Sons, Lescher & Webb Ltd., Liverpool*). "Promptly relieves coughs, colds, catarrh, sore throats and huskiness." *Formula*:—Terebentum 0.33%, Menthol 0.16%, Eucalyptol 0.03%, Ol. Menth. Pip. 0.014%, Potassii Chloras 1.278%, Borax 1.279%, Glycerin 0.255%, Basis ad 100.

Ex-Lax (*Ex-Lax Ltd., Slough*). "Chocolate laxative for complete constipation relief." *Formula*:—Chocolate 90%, Phenolphthalein 10%. *Dose*: 1 or 2 tablets.

Falconer's Golden Compound (*Falconer & Co. Ltd., London*). "It cures lumbago in two hours in nearly all cases." *Formula*:—Sapo Mollis .5%, Ol. Arach., .375%, Ol. Ricin. .375%, Ol. Terebinth. 2.5%, Alcoh. sol. extract of 16% Capsic. Sp. Meth. Indust., ad 100%.

[P1] **Famel Brand Syrup** (*Optrex Ltd., Greenford*). "Affections of the respiratory organs: chronic bronchitis, coughs, neglected colds, catarrh, asthma, influenza." *Formula*:—Purified Creosote B.P. 0.13 gm., Calcium Lactophosphate 0.125 gm., Guaiacol B.P. 0.065 gm., Codeine 0.023 gm., Tinct. Aconite B.P.C. 0.55 gm., Flavour and Syrup ad 100 gm., contains 0.00027% w/w alkaloids of aconite calculated as aconitine. *Dose*: Adults 1 dessertspoonful; Children, $\frac{1}{2}$ to 1 small teaspoonful or less according to directions.

Family Lung Syrup (*P. H. Galloway Ltd., London*). "Pleasant to take and perfectly safe for children and adults." *Formula*:—Ol. Anisi 0.60, Ext. Ipec. Liq. 0.68, Chlorof. 510, Acid Acetic 3.000, Acetum Scillæ 2.675, Syr. Scillæ 6.000, Spt. Ether Chlor. 500, Sacch. Ust. 1.800, Syrup ad 100.000. *Dose*: Children, $\frac{1}{2}$ to 2 teaspoonfuls; Adults, 1 or 2 tablespoonfuls.

[P1] **Fellows Compound Syrup of Hypophosphites** (*Fellows Medical Manufacturing Co. Ltd., Montreal*). Each fluid drachm contains Manganese Hypophosphite $\frac{1}{2}$ gr., Potash Hypophosphite $\frac{1}{2}$ gr., Soda Hypophosphite $\frac{1}{2}$ gr., Iron $\frac{1}{2}$ gr., Lime $\frac{1}{2}$ gr., Quinine $\frac{1}{2}$ gr., Strychnine $\frac{1}{2}$ gr.

Fellows Laxative Tablets (*Fellows Medical Manufacturing Co. Ltd., Montreal*). "Fellows Laxative Tablets are a corrective for habitual constipation due to intestinal inactivity or to an insufficiency of the digestive secretions." *Formula*:—Each tablet contains: Extract of Cascara Sagrada grm. 0.049, Aloin grm. 0.008, Podophyllin grm. 0.0065. *Adult dose*: 1 or 2 tablets.

Felsol Brand Powders (*British Felsol Co. Ltd., London*). "For asthma." *Formula*:—Phenazone 0.47, Iodopyrine 0.03, Antipyrine Aceto-salicylas 0.40, Caffeine 0.10, Ext. Viscum Album 0.01, Ext. Brachycladii 0.01.

Fennings' Adult Cooling Powders (*The Trustees of Alfred Fennings, Cowes, I.O.W.*). "For cooling all feverish heats of the blood and for colds, coughs, eruptions and headaches." *Formula*:—Pot. Chloras 58%, Glycyrrh. 5%, Ath. 8%, Powdered Malt 24%, Mag. Carb. Lev. 5%. *Dose*: 1 powder.

Fennings' Children's Cooling Powders (*The Trustees of Alfred Fennings, Cowes, I.O.W.*). "For children cutting their teeth. For feverishness, colds, thrush, eruptions, sickness and disordered bowels." *Formula*:—Pot. Chloras 30%, Glycyrrh. 33%, Powdered Malt 33%, Mag. Carb. Lev. 4%. *Dose*: 1 powder.

Fennings' Fever Cure (*The Trustees of Alfred Fennings, Cowes, I.O.W.*). "The celebrated remedy for prevention and cure of whooping cough, influenza, colds, measles, mumps, chicken pox, sore throat, cholera, typhus, low fever, diphtheria, dysentery, smallpox, scarlet fever, bowel complaints, griping pains, windy spasms, bile diarrhoea, looseness and fluxes, etc." *Formula*:—Ac. Nitric B.P. 1.53%, Spiritus Vinus Rect. 27%, Oil Menth. Pip. 0.075%, Pulv. Sang. Drag. 0.048% w/v, Aqua ad 100 v/v.

Fennings' Little Healers (*The Trustees of Alfred Fennings, Cowes, I.O.W.*). "For coughs and colds." *Formula*:—Ipecac 90%, Conf. Ros. Can. 10%. *Dose*: 1 pill.

Fennings' Ointment (*The Trustees of Alfred Fennings, Cowes, I.O.W.*). "For all irritations of the skin, eczema, dermatitis, chilblains, boils, impetigo, nettlerash, psoriasis, infantile eczema, burns and scalds." *Formula*:—Zinc Oxide 4%, Calamine 3%, Liq. Picis Carbonis 6%, Balsam of Peru 3%, Borneyl Acetate 1%, Adeps Lan. 6%, Paraff. dur. 12%, Paraff. Moll. to 100%.

Fennings' Rheumatic and Erysipelas Drops (*The Trustees of Alfred Fennings, Cowes, I.O.W.*). "For rheumatism, lumbago, erysipelas, rheumatic gout, sciatica, etc." *Formula*:—Ammon. Carb. 4.73% w/v, Sacch. Ust. 59%. Aqua ad 100 B/V. *Dose*: 1 teaspoonful.

Fennings' Stomach Strengtheners (*The Trustees of Alfred Fennings, Cowes, I.O.W.*). "Pills to assist digestion, prevent acidities, wind and spasms, to remove heartburn, palpitations and pain after eating, and to enable the stomach to convert food into proper nourishment for the body." *Formula*:—Rheum. 23%, Sap. Dur. 24%, Ipecac. 53%. *Dose*: 1 pill.

Fennings' Whooping Cough Powders (*The Trustees of Alfred Fennings, Cowes, I.O.W.*). *Formula*:—Glycyrrh. 28%, Sulphur. Præcip. 68.5%, Alum Exsic. 3.5%. *Dose*: 1 powder.

Fennings' Worm Powders (*The Trustees of Alfred Fennings, Cowes, I.O.W.*). "For safely destroying worms: The Tape Worm, Round Red Worm, and Thread Worm." *Formula*:—Lactos 33%, Sulphur Præcip. 67%. *Dose*: For children, 1 powder; for adults, 3 powders.

Ferocal (Squire's Chemical Food) (*Squire & Sons, London*). *Formula*:—Active constituents in each fluid drachm: Anhydrous Iron Phosphate 0.64 grain, Calcium Phosphate 1.80 grain, Potassium Phosphate 0.08 grain, Sodium Phosphate 0.06 grain. *Dose*: $\frac{1}{2}$ to 1 teaspoonful in water.

✓ **Fersolate Tablets** (*Glaxo Laboratories Ltd., Greenford*). *Formula*:—Each tablet (3 gr. Ferr. Sulph. exsic.) contains Iron (ferrous) 1 gr., Copper $\frac{1}{100}$ gr., Manganese $\frac{1}{100}$ gr. *Dose*: 1 tablet.

Firth's Green Salve (*H. Firth, Liverpool*). "Cures poisoned wounds, bad legs, boils, whitlows, carbuncles, burns, scalds, cuts, and all forms of gatherings and eruptions." The composition of this preparation was not disclosed.

Fissan Brand Anal Ointment (*Genatosan Ltd., Loughborough*). "For the treatment of piles, anal pruritus and fissures." *Formula*:—Lactalbumin 1.0, Fluoro-Silica Colloid (Fissan Ltd.) 0.4, Colloid silicic acid 0.4, Colloidal kaolin 3.2, Bismuth subnitrate 0.5, Bismuth salicylate 0.05, Menthol 0.1, Boric acid 3.0, Zinc oxide 9.0, Wool fat ointment 82.0, Solution of hamamelis 0.2, *p*-oxybenzoic methyl ester 0.15.

Fissan Brand Anal Suppositories (*Genatosan Ltd., Loughborough*). "Indications: Hemorrhoids, anal pruritus, and fissures." *Formula*:—Lactalbumin 1.0, Fluoro-Silica Colloid (Fissan Ltd.) 0.3, Colloidal kaolin 1.0, Bismuth subnitrate 1.0, Bismuth salicylate 1.0, Menthol 0.1, Boric acid 1.0, Zinc oxide 4.0, Wool fat ointment 3.0, Spermaceti 30.0, Oil of theobroma 55.0, Solution of hamamelis 2.6.

Fissan Brand Paste (*Genatosan Ltd., Loughborough*). "For treatment of irritation, sores, cuts, abrasions, burns and scalds." *Formula*:—Lactalbumin 1.0, Fluoro-Silica Colloid (Fissan Ltd.) 0.15, Colloidal silicic acid 0.6, Colloidal kaolin 3.0, Boric acid 3.0, Bismuth Subnitrate 0.5, Zinc oxide 9.5, Methyl Ester of *p*-hydroxybenzoic Acid 0.15, Wool fat 53.4, Liquid Paraffin 12.3, Water 16.4.

✓ **Fitilin Brand Revitalising Rub** (*London Solvents Ltd., London*). "A muscle and nerve stimulant which restores elasticity and tone to tired muscles and jaded nerves." *Formula*:—Sod. Salicyl 22680 Cgms., Ethyl Salicyl 1134 Cgms., Ol. Gauthier 425 Cgms., Ol. Chierianthi 71 Cgms., Ol. Verbenæ 142 Cgms., Camphoræ 567 Cgms., Ac. Acetylsal. 5192 Cgms., Ol. Ricini 22680 Cgms., Terebinth 850 Cgms., Ol. Heliotrop 18 Cgms., Ol. Cedri 213 Cgms., Terpineol 567 Cgms., Ol. Terebinth 5670 Cgms., Sucros Octa Acetatis 2268 Cgms., Æthylis Phthalatis 4620 Cgms., Sp. Meth. Indust. 22273 mils.

Formitrol Brand Formalin Pastilles (*A. Wander Ltd., London*). "An efficient mouth and throat antiseptic." *Formula*:—Formaldehyde gr. $\frac{1}{2}$, Menthol gr. $\frac{1}{16}$, Citric Acid gr. $\frac{1}{8}$, Sugar Base q.s.

[P1] **Freeman's Chlorodyne** (*Freeman's Chlorodyne Ltd., London*). The poisonous ingredients are stated to be Ext. Opii Liq. (10% morphine, anhydrous) 1.5%, Codeina Phosph. 0.22%, Chloroform 6%. Other ingredients include Ether 1.5% and Proof Spirit 1.7%.

Freezone Brand Corn Remover (*W. L. Dodge Ltd., London*). "Makes corns, calluses and hard skin disappear." *Formula*:—Terebinth Canadens 5.5%, Ol. Ricini 3.5%, Zinc. Chlor. 2%, Acid Salicyl 14%, Collodium 75%.

Fructolax (*Savory & Moore Ltd., London*). "Deliciously-flavoured Hydrocarbon Jelly Laxative." *Formula*:—Soft Paraffin 80%, Sugar 15%, Colour and Flavouring 5%. *Dose*: Children, $\frac{1}{2}$ to 1 teaspoonful; Adults, 1 to 3 teaspoonfuls.

Fructole Carpinæ Co. (Mayfair A Brand) (*Savory & Moore Ltd. London*). *Formula*:—Sod. Brom. 1.78%, Ammon. Brom. 1.78%, Potass. Brom. 8.89%, Pilocarpine Nitrate 0.00622%, in a flavoured syrup and glycerin base. *Dose*: $\frac{1}{2}$ to 1 tablespoonful.

Fruligar (*The manufacturer's name is not given on the container*). "The new garlic cough cure. A positive remedy for coughs, bronchitis, asthma, croup, catarrh, whooping cough, children's coughs." *Formula*:—Pot. Iod. 1.09, Ext. Glycyrrhiz. Liq. 5.00, Ext. Ipecac. Liq. .25, Sod. Benz. .10, Ext. Scillæ Liq. .25, Formyl Terchlor. .50, Sacch. Ust. q.s., Ol. Menth. Pip. .05, Ol. Alii Satir. .008, Syr. ad 100. *Dose*: Children, $\frac{1}{2}$ to 2 teaspoonfuls; Adults, 1 to 2 teaspoonfuls.

Fuller Brand Celery Perles (*Lowthers Ltd., London*). "For rheumatic ailments." *Formula*:—Each perle contains the following ingredients: Solid Extract prepared from fresh Celery Seed 1.20 grn., Solid Extract Buchu .20 grn., Phenolphthalein .20 grn., Solid Ext. Cimicifugæ .10 grn., Sulphur Sub. .40 grn., Sodii Sal. .90 grn. "Our formula No. 12640." *Dose*: 2 perles.

✓ **Fynnon Salt** (*Fynnon Ltd., Brentford*). "Famous Saline Treatment. Nature's Salt of the Earth. For rheumatism, gout, sciatica, neuralgia, lumbago, constipation, liver, kidneys, lassitude, etc." *Formula*:—Sod. Sulph. 95.96%, Sod. Bicarb. 1.95%, Pot. Sulph. 2.05%, Lith. Sulph. 0.033%, and traces of iron and sodium chloride. *Dose*: 1 teaspoonful in water.

✓ **G.S. Brand Tablets** (*Westminster Laboratories Ltd., London*). "The natural internal cleanser. For rheumatism, slimming and constipation." *Formula*:—Sodii Sulphas Exsicc. gr. 4½, Phenolphthalein gr. ¼, Potassii Iodid gr. ½, Zingiber gr. ½, Kaolin q.s. *Dose*: 1 tablet.

✓ **Galloway's Family Cough Syrup** (*P. H. Galloway Ltd., London*). "For coughs, colds, hoarseness, bronchitis and whooping cough." *Formula*:—Ol. Ment. Pip. .008, Ol. Anisi .060, Ext. Ipec. Liq. .090, Chlorof. .575, Acid. Acetic. 3.000, Syr. Scillæ 6.000, Acetum Scillæ 2.675, Spt. Ether Chlor. .500, Sacch. Ust. 1.800, Syrup ad 100.000. *Dose*: Adults, 1 or 2 tablespoonfuls.

✓ **Gar Brand Garnette Antiseptic Remedy** (*H. E. Matthews Ltd., London*). "The sovereign cure for all diseases of the skin." *Formula*:—Cera Flav. 4.1%, Hydrarg. Ammoniatum 2.05%, Liq. Picis. 8.2%, Paraffin Molle 83.24%, Essential Oils to perfume 2.41%.

✓ **Genasprin** (*Genatosan Ltd., Loughborough*). "The safe brand of aspirin. For headache, influenza, colds, toothache, gout, neuralgia, nerve pains, neuritis, rheumatism, and uric acid disorders." *Formula*:—Acetyl Salicylic Acid 5 grs., Arrowroot 1.25 grs. *Dose*: 2 tablets.

✓ **George Brand Gravel Pills** (*J. O. George, Hirwann, Glam.*). *Formula*:—P. Podoph. Res. 0.1, P. Ginger 3.2, P. Gamboge 1.2, P. Jalapæ 9.6, P. Coloc. Pulp. 0.4, Soda Carb. Ex. 3.2, Saponis 14.9, Aloes Barb. 38.4, Ol. Juniper 0.06, Hexamine 0.02, Coating to 100. *Dose*: 2 pills.

✓ **George Brand Pile and Gravel Pills** (*J. O. George, Hirwann, Glam.*). "Universally held in esteem." *Formula*:—P. Podoph. Res. 0.2, P. Ginger 3.2, P. Gamboge 3.2, P. Jalapæ 9.6, P. Coloc. Pulp. 0.6, P. Saponis 12.8, P. Aloes Barb. 38.4, Ol. Ment. Pip. 0.03, Ext. Cascara 0.6, P. Ipecac. Ver. 0.3, Hexamine 0.03, Coating to 100. *Dose*: 2 pills.

✓ **George Brand Pills for the Piles** (*J. O. George, Hirwann, Glam.*). "Universally held in esteem." *Formula*:—P. Podoph. Res. 0.2, P. Ginger 3.2, P. Gamboge 1.07, P. Jalapæ 9.6, P. Coloc. 0.53, P. Aloes Barb. 38.4, Saponis 12.8, Ol. Carui 0.07, Coating to 100. *Dose*: 1 pill.

✓ **Germicidal Soap P.D. & Co.** (*Parke Davis & Co., London*). "For dermatological and surgical use. Containing 2% (w/w) of Mercuric Iodide."

✓ **Germolene** (*Veno Drug Co. Ltd., Manchester*). "Aseptic ointment. Soothing and healing. Valuable as an emollient embrocation or skin dressing." *Formula*:—Adeps Lanæ 53.2%, Paraffinum Molle 26.601%, Amylum 7.98%, Zinci Oxidum 7.99%, Ethylis Salicylas 3.011%, Phenol 1.202%, Menthol 0.012%, Rubrum Scarlatinum 0.004%.

✓ **Germolene Brand Blood Purifier and Tonic** (*Veno Drug Co. Ltd., Manchester*). "Prepared from ingredients known to have exceptional restorative value in the treatment of impure blood, anæmia, pimples, abscesses, ulcers, boils, bad legs, eczema, rashes and other blood impurities." *Formula*:—Dec. Sarsæ Co. Conc. 16.25, Mist. Sennæ Co. 37.5, Pot. Iodid. 0.45, Ferri et Ammonii Citras 2.05, Syrupus 31.25, Aq. Chlorof. ad 100.00. *Dose*: Adults, 2 teaspoonfuls; Children, 1 teaspoonful; Children under 10 years of age, ½ teaspoonful.

✓ **Germolets Brand Tablets** (*Veno Drug Co. Ltd., Manchester*). "Containing therapeutic reagents of acknowledged value in the treatment of impure blood, anæmia, pimples, abscesses, ulcers, boils, bad legs, eczema, rashes and other cutaneous and blood disorders." *Formula*:—Berberis Aquifol. 17.857, Echinac. Angus. 17.857, Cal. Phos. 35.714, Acacia Gum 7.120, Sacrose 17.857, Ferri Glycerophos. 1.785, Salicinum 0.223, Mag. Silic. Nat. 1.567, Ol. Aurant. T'pless 0.20. *Dose*: 1 or 2 tablets.

✓ **Germoloids Brand Suppositories** (*Veno Drug Co. Ltd., Manchester*). "A remedy for the internal treatment of hæmorrhoids." *Formula*:—Zinci Oxidum 8.00%, Ethyl Hydroxybenzoas 2.85%, Resorcinol 2.14%, Bismuthi Subchloridum 5.71%, Toluene Azo-Toluene-Azo-β-Naphthol 0.007%, Ol. Theobromatis ad 100%.

✓ **Gilley's (Dr.) Herbal Laxative** (*Gilley's Laboratories, London*). "A nature aid composed of aromatic herbs. An intestinal cleanser and system purifier." *Formula*:—Frangula Cortex 23.07%, Psyllium Semni 24.61%, Sennæ Fol. 46.18%, Anisi Fructi 3.07%, Sassafras Cortex 3.07%. *Dose*: One-third of a teaspoonful.

✓ **Glow-Well Liniment** (*Evans, Sons, Lescher & Webb Ltd., London*). "Produces a warm glow which relieves the pain of rheumatism, sciatica, lumbago, sprains, etc." *Formula*:—Bacc. Capsici 10 unit, Essent. Oil of Camphor 77 unit, Ol. Succini Rect. 3.75 units, Ol. Terebinthinæ 19.2 units.

✓ **Glycolactophos** (*Roberts & Co., London*). "Its use is indicated in neurasthenia, neuralgia, wasting, dyspepsia, anæmia, insomnia, rickets, nervous

disorders and during convalescence." *Formula*:—Glycerophosphate of Sodium 2½%, Glycerophosphate of Calcium 2½%, Casein 95%. *Dose*: ½ to 2 teaspoonfuls.

Glyco Thymoline Brand (*Kress & Owen Ltd., Farnham*). "Alkaline deodorizing and non-irritating solution." *Emergency formula*:—Active constituents: Sodium Borate 2.084, Thymol 0.044, Menthol 0.044, Sod. Salicyl 0.095, Sod. Bicarb 2.084, Sp. Vin. Rect. 3.605, Ol. Pini Pumil 0.044, Cajeputul 0.088, Ol. Betula 0.035, all per cent.

Grasshopper Brand Ointment (*Grasshopper Ltd., London*). "For the treatment of bad legs, simple ulcers, abscess, carbuncle, boils, piles, scalds, burns, insect stings, ringworm, broken and unbroken chilblains." *Formula*:—Resina 31.68, Cera Flav. 7.94, Oleoresina Laricis 23.74, Ol. Oliv. 15.84, Paraff. Moll. Alb. 19.81, Cupri Acetas 0.99.

Greenfield's (Dr.) Whooping Cough Mixture (*Wyley's, Coventry*). *Formula*:—Syr. Rubri Idæi 25.000%, Scilla Glycosida (Scillitin: Scillidiuretin) = Tinct. Scillæ 2.085%, Tinct. Cardam. Co. 2.085%, Acid. Nitrici Dil. 2.085%, Chloroformum 0.217%, Spt. Thymi Rubri 0.072%, Aq. Destillatæ 68.456%.

Gripe Water Brand Carminative (*W. Woodward Ltd., London*). "For convulsions, gripes, acidity, flatulence, whooping cough, and the distressing complaints incidental to infants at the period of cutting their teeth, allaying the pain, giving instant relief and rendering this crisis perfectly mild and free from danger." *Formula*:—Ol. Anethi. m. 2, Sodii Bicarbonas gr. 20, Spiritus Rectificatus m. 94, Syrupus Simp. fl. dr. 9½, Aqua Dest to fl. oz. 4½. *Dose*: 1 to 3 teaspoonfuls, according to age.

Guaranteed Acetic Acid (*F. Coutts & Sons, London*). "For application with flesh brush or sponge." "The Acid Cure." *Formula*:—20 to 25% Real Acetic Acid.

Guy's Brand Tonic (*Guy's Tonic Ltd., London*). "Such splendid results have invariably followed the use of Guy's Brand Tonic that we feel justified in recommending it as a thoroughly reliable remedy for disorders of the stomach, liver and nerves and in all cases of debility and loss of strength." *Formula*:—Gentianæ Radix 0.178, Aurantii Cortex Recens 0.067, Cardamomi Semina 0.022, Coccus Cacti 0.178, Spiritus Chloroformi 3.58, Acidum Phosphoricum 0.033, Acidum Glycerophosphoricum 0.033, Acidum Nitricum 0.123, Acidum Hydrochloricum 0.164, Aqua ad 100.0. *Dose*: 1 tablespoonful; Children, 1 teaspoonful to 1 dessertspoonful.

Guy's Fruit Pills (*Guy's Tonic Ltd., London*). "The best family aperient." *Formula*:—Potassii Sulphas 4.0, Oleoresina Zingiberis 0.5, Zingiber 0.5, Colocynth 4.0, Aloe 8.0, Ipomœa 8.0, Oleum Cari 1.0, Excipient ad 100.0. *Dose*: 2 or 3 pills.

Hæmovin (*Moore Medicinal Products, Aberdeen*). "Ointment for hæmorrhoids, pruritus and anal fissures." *Formula*:—Carvasept. 0.01 g., Ammon. sulf. ichthyn. 0.30 g., Menthol reconst. 3.00 g., Ungt. Lanæ 76.49 g., Vasel. Flav. 2.20 g., Ol. Arachides 18.00 g.

Hair (Dr.) Brand Asthma Cure (*Dr. Hair's Asthma Cure Ltd., Staines*). "The constitutional remedy. The standard treatment for bronchitis and asthma." *Formula*:—Sodii Benzoas 0.625, Trichloromethane 0.46, Sodii Iodidum 0.3, Potassii Iodidum 5.5, Spiritus Tenuior 9.8, Pix Liquida 0.01, Aqua Destillata ad 100.

Hair (Dr.) Asthma Cure Pastilles (*Dr. Hair's Asthma Cure Ltd., Staines*). "Asthma and bronchitis treatment." *Formula*:—Pot. Iod. 14.6%, Sod. Benz. 0.835%, Sod. Iod. 0.70%, Chloroform 0.15%, Succ. Glycyrrh. 2.1%, Glusidum 0.105%, Pix Liquida (equiv.) 0.09%, Ess. Blackcurrant 3.95%, Sacch. Ust. 1.05%, Pastille Base to make 100%. *Dose*: 1 to 4 pastilles.

Hair (Dr.) Brand Bronchial Cough Remedy (*Dr. Hair's Asthma Cure Ltd., Staines*). "This medicine is so effective that, in increased doses, it is sometimes found to suit asthmatic and bronchitic sufferers instead of the 'Asthma Cure'." *Formula*:—Potassii Iodidum 1.1%, Ext. Stillingia (1 in 1) 6.25%, Ext. Yerba Santa (1 in 1) 8.25%, Ext. Grindelia Robusta (1 in 1) 11.1%, Chloroform 0.5%, Liq. Ammon. Dil. 1.8%, Sod. Hydrox. 0.125%, Aq. Destillata ad 100. *Dose*: 1 teaspoonful; Children, ½ or ⅓ teaspoonful.

[P1] Hair (Dr.) Brand Catarrh Cure Pills (*Dr. Hair's Asthma Cure Ltd., Staines*). "For constitutional treatment of catarrh." *Formula*:—Each pill contains Quininæ Sulphatis gr. 1.75, Ext. Hyoscy. Sicc. B.P. gr. 0.75, Ext. Gentianæ Rad. gr. 0.75.

Hair (Dr.) Brand Liver Pills (*Dr. Hair's Asthma Cure Ltd., Staines*). "Vegetable only. For constipation, headaches, indigestion, stomach disorder, biliousness, lassitude, boils and pimples, complexion." *Formula*:—Podophylli Resina gr. $\frac{1}{2}$, Sapo durus pulv. gr. $\frac{1}{16}$, Glycerrhizæ Rad. gr. $\frac{1}{16}$, Syr. Glucosi Liq. gr. $\frac{1}{2}$. *Dose*: 1 pill.

Halborage (*Allen & Hanburys, London*). "The nicest way of taking halibut-liver oil. Contains the growth-promoting Vitamin A, and the Antirachitic Vitamin D, with the Antiscorvy Vitamin C." *Formula*:—Ol. Hippogloss. B.P. 3.0, Calciferol 0.0006, Succ. Aurant. Conc. 25% w/w. *Dose*: $\frac{1}{2}$ to 2 teaspoonfuls.

Halmagon Brand Tablets (*Tonicity Laboratories Ltd., London*). "A scientific combination of nature's mineral compounds for use as a daily ration to promote and maintain maximum metabolic activity." *Formula*:—Magnesii Chloridi .395 gram, Magnesii Bromidi .0133 gram, Magnesii Iodidi .000067 gram, Magnesii Fluoridi .0006 gram, Excipient q.s. = .45 gram. *Dose*: 3 tablets.

Haratox Brand Tablets (*Haratox Ltd., Manchester*). "For the treatment and prevention of rheumatism, gout, lumbago, sciatica, neuritis and gallstones." *Formula*:—Rudbeckia-Pal $\frac{1}{2}$ gr., Carbamide—CO, (N.H.2)2 (Trit) .0001 $\frac{1}{2}$ gr., Thuja-Occ. Trit. .0001 $\frac{1}{2}$ gr., Sulph-Sub. 1 gr., Guacum 1 gr., Sach. Lac. and Oryza-Sat q.s. *Dose*: 1 tablet.

Harley's Three Salts (*Thos. Harley Ltd., Perth*). "For rheumatism, gout, constipation, stomach, liver, and kidney ailments. Safe for slimming." *Formula*:—Mag. Sulph. Exsicc. 55%, Sod. Sulph. Exsicc. 42.3%, Pot. Tart. Acid. 1.7%, Pot. Sulph. c. Sulph. 1%. *Dose*: $\frac{1}{2}$ to 1 teaspoonful; Aperient, 1 to 2 teaspoonfuls.

[P] **Harrison's Pomade** (*G. W. Harrison Ltd., Reading*). "A certain cure for nits and vermin in the hair." Contains .4% w/w veratrine.

Healex Skol Brand Healing Antiseptic (*Skol Products Ltd., Worcester*). "For eczema and all skin infections, irritations, cuts, bites, chilblains, burns. An antiseptic gargle and mouth-wash." *Formula*:—Acidum Tannicum 5.00, Acidum Carbolicum 0.50, Menthol 0.25, Glycerinum 3.00, Phenyl Salicylate 1.00, Alcohol et aqua ad 100.00.

Heath and Heather's Catarrh Pastilles (*Heath & Heather Ltd., St. Albans*). "Cure coughs, colds, hay fever, bronchitis." *Formula*:—Creosote $\frac{1}{2}$ oz., Menthol 1 oz., Pine Oil 1 oz., Pastille Base to 10 lb.

Herbalene (Lusty's) (*Lusty's Natural Products, Westcliff-on-Sea*). "The safe tonic laxative." *Formula*:—Sennæ Folium, Foeniculum, Frangula, Mate Folia, Sambucus, Chelone Glabra, partes equales.

Herbile Pills (*W. F. Howard, London*). "May be regarded as supplying what is deficient in the system rather than as a drug to purge." *Formula*:—Aloes Barb. gr. 1 $\frac{1}{2}$, Sacch. Lact. gr. 1 $\frac{1}{2}$.

Hinksman's Asthma Cigarettes (*Hinksman & Forrest Ltd., Carlisle*). "For asthma, bronchitis, whooping cough, shortness of breath, etc." *Formula*:—Contains Lobelia 2% and Stramonium 7.2%.

Hinksman's Asthma Reliever (*Hinksman & Forrest Ltd., Carlisle*). "For asthma, bronchitis, whooping cough and shortness of breath." *Formula*:—Contains Lobelia 2% and Stramonium 72%.

Hinksman's Asthma Smoking Mixture (*Hinksman & Forrest Ltd., Carlisle*). "For the immediate relief of asthma, bronchitis, whooping cough, shortness of breath, etc." *Formula*:—This preparation contains Lobelia 2% and Stramonium 72%.

Hockin's Remedy (*R. A. Hockin, Ryde, I.O.W.*). "For asthma and bronchitis." "It has great advantages over medicines taken into the stomach, coming at once into contact with the Bronchial Tubes and Lungs; hence all injury to digestive organs is avoided." *Formula*:—74/100 Pulv. Stramonii.

Holdroyd's Gravel Pills (*J. Holdroyd, London*). "The great cure for gravel, lumbago, backache, wind and water ailments, and all disorders of the kidneys." *Formula*:—Sod. Bicarb. 16.9%, Rhei Rhizoma 28.8%, Sap. Dur. 26.0%, Ol. Juniper Lig. 6.5%, Ol. Anisi 6.5%. *Dose*: 2 pills.

Holloway's Brand Ointment (*Holloway's Pills Ltd., London*). "For the treatment of ulcers, cuts, chilblains, chapped hands, wounds, burns, bruises, insect bites, boils, bad legs, etc." *Formula*:—Cera Flav. 19.20%, Tereb. Venet. Fact. B.P.C. 28.80%, Butter Fat 52.00%.

Holloway's Brand Pills (*Holloway's Pills Ltd., London*). "Act by purifying the system, regulating the functions, harmonising the bodily workings,

enriching and cleansing the blood, toning liver, kidneys and excretory organs, ensuring full nourishment from your food, strengthening the whole body." *Formula*:—Aloe 36.15%, Pulvis Zingiberis 36.15%, Jalapa Pulverata 12.00%, Cambogia 12.00%, Sapo Durus 3.70%. *Dose*: 2 to 6 pills.

Homoea Brand Ointment (*Nunol Ltd., Newcastle-on-Tyne*). "Use for scalds, burns, aches, wounds and neuralgia." *Formula*:—Ol. Cocois 20.82, Paraff. Moll. 26.02, Paraff. Dur. 26.02, Cera Alb. 7.29, Adeps. 2.08, Camph. 2.08, Ol. Cajaputi 2.42, Ol. Terebinthi. 8.98, Ol. Rosmarini. 0.585, Ol. Eucalypti 0.585, Liq. Am. Fort. 1.56, Aqua Pura 1.56.

Hooper's (Dr. John) Female Pills (*Dr. John Hooper's Female Pills Co. Ltd., Reading*). *Formula* (*in grains*):—Each pill contains Canillæ $\frac{1}{2}$, Pepper Long $\frac{1}{2}$, Hellebor Nig. $\frac{1}{2}$, Myrrh $\frac{1}{2}$, Aloes $\frac{1}{2}$, Senna Alex $\frac{1}{2}$, Ferri Sulph. Ex. $\frac{1}{2}$. *Dose*: 2 or 3 pills.

Hoyle's Pure Vegetable Viscous Oil (*Hoyle's, Liverpool*). "For rheumatism, sciatica, constipation, digestive and gastric complaints, also sprains, bruises, swollen knees and feet." *Formula*:—Ol. Line 98%, Ol. Aurant. 1%, Ol. Limonies 1%. *Dose*: 1 dessertspoonful.

Hypotensyl "Opocrin" Brand (*The Anglo-French Drug Co. Ltd., London*). "The active principles of viscum (Gui) with gland extracts for the treatment of conditions associated with high blood pressure." *Formula*:—Each tablet contains Viscum Extract 0.075 gm., Hepatic Extract 0.10 gm., Pancreatic Extract 0.05 gm. *Dose*: 3 to 6 tablets.

Hytex Pile Balm (*Knox Laboratories, London*). "A modern and scientifically produced treatment that usually stops the pain from piles in 15 to 20 minutes." *Formula*:—Tannic Acid 3%, Quinine Urea HCl. 4%, Benzoic Acid 2%, Menthol 1%, Paraffinum M.F. 65½%, Adeps. Lanæ 28½%.

Ideal Warming Liniment (*British Drug Houses Ltd., London*). "A household remedy for rheumatism, lumbago, sciatica, neuralgia, sprains, also for bronchitis, colds and coughs." *Formula*:—Ol. Sassafras 3, Capsicin 3, Ol. Myrist. 7, Ol. Succini 72, Ol. Camph. 246.

Idozan Brand Colloidal Iron (*Coates & Cooper Ltd., London*). "5% Fe. Specially indicated in the treatment of anæmia and allied conditions. Does not constipate, disturb digestion or discolour the teeth." *Formula*:—Colloidal Iron Solution 84%, Pure Alcohol 5%, Sugar 10%, Flavouring 1%. *Dose*: 1 teaspoonful to 1 tablespoonful.

Iglodine (*Iglodine Co. Ltd., Newcastle-upon-Tyne*). "The antiseptic for internal and external use." *Antiseptic Formula*:—Each fluid oz. contains Phenol 0.39 grain, Pot. Monoxide (K₂O) 0.04 grain, Combined Iodine 0.19 grain.

Iglodine Antiseptic Ointment (*Iglodine Co. Ltd., Newcastle-on-Tyne*). "Heals like magic; leaves no scar." *Formula*:—Paraff. Moll. Flav. 76%, Adeps. Lan. 4%, Pot. Monoxide 0.05%, Combined Iodine 0.15%, Phenol 0.36%, Alcohol w/v 0.18%.

Indian Cerate (*Reade Brothers & Co. Ltd., Wolverhampton*). "The most efficacious, agreeable and useful remedy known." *Formula*:—Camph 0.5, Zinc Oxid 3.7, Acid Boric 1.6, Phenol Liq. 0.27, Ol. Arach 21, Adeps Lan Hydros 9.1, Cetac 17.7, Paraff Moll Alb, etc., ad 100%.

[P2-87] **Inecto Rapid** (*Rapidol Ltd., London*). "No. 8½ Golden Blonde. (Six other shades supplied.) The guaranteed permanent hair colouring. Inecto Rapid is sold on the express condition that the manufacturers, Rapidol Ltd., and the immediate vendor, are not liable for personal injury, illness or damage resulting from its use." *Formula*:—Supplied in two separate bottles. Solution A "contains 0.2% w/v phenylene diamine."

Infants' Friend (*Potts' Infants' Friend Ltd., Nottingham*). *Mist. Mag.* Hydrox. 12.5%, Sodii Brom. 2.5%, Sacrosum 43.269%, Ext. Rhei Liq. B.P.C. 0.1%, Ol. Anethi 0.125%, Ol. Coriand. 0.006%, Aq. Anethi 41.5%. *Dose*: 10 drops to a teaspoonful.

Iodex Brand (*Menley & James Ltd., London*). "May be used externally with advantage in the many conditions requiring an antiseptic, penetrating and inflammation-reducing agent." *Formula*:—Resublimed iodine 4% in neutral, parogen, petrolatum base.

Iodex Brand Suppositories (*Menley & James Ltd., London*). "Antiseptic. Inflammation-reducing, absorptive. Indicated in hæmorrhoids and other inflammatory rectal conditions. They should be used strictly in accord with the advice and instructions of the physician." *Formula*:—Resublimed iodine (4%) in a neutral, parogen petrolatum base, in gelatine envelope.

Iodex cum Methyl Salicyl (*Menley & James Ltd., London*). "May be used externally with advantage in many conditions requiring an analgesic,

antiseptic, penetrating and inflammation-reducing agent." *Formula*:—Resublimed Iodine (4%) c. Methyl Salicyl (5%) in a neutral parogen, petrolatum base.

Iodex (Liquid). (*Menley & James, Ltd., London*). "Resublimed iodine (2%) in pure vegetable oil. For aural, naso-pharyngeal and other uses."

Iodex Pessaries (*Menley & James Ltd., London*). "Antiseptic. Inflammation-reducing. Absorptive. They should be used strictly in accord with the instructions of the physician." *Formula*:—Resublimed iodine (4%) in a neutral parogen petrolatum base, in gelatine envelope.

Iodine Medol Brand Aseptic Ointment (*Pearson's Medol Ltd., London*). "For cuts, wounds, burns, scalds, chilblains, rashes, erysipelas, ringworm, eczema, etc." *Formula*:—Iodum 1.00%, Ol. Ricini 23.75%, Cresol 2.25%, Paraffinum Liq. 49.65%, Sodii Hydras. 1.10%, Ceresin 17.75%, Liq. Ammon. Fort. 1.75%, Aqua Dest. 2.75%.

Iodised Throat Loz. **Smith** (*Smith & Co., London*). "The throat antiseptic." *Formula*:—Liq. Iodi Mit. m. $\frac{1}{2}$, Liq. Formaldehyde m. $\frac{1}{4}$, Phenol gr. $\frac{1}{16}$, Menthol gr. $\frac{1}{32}$, Acid. Citric. gr. $\frac{1}{16}$, Basis Troch., Gum. Acac., Sacch. Purif., etc., q.s.

Ionised Iodine (Molson Brand) (*The Molson Ionised Iodine Co. Ltd., Maidstone*). "Painless, non-poisonous, penetrating antiseptic and germicide. Proved by demonstration bacteriologically and clinically. Kills staphylococci, streptococci and virulent faecal bacilli coli in 2½ minutes. Far better than Tincture of Iodine for cuts, burns, scalds, contusions and wounds of every description." *Formula*:—Iodine 7.91 parts, Chlorine 10.04 parts, Potassium 2.89 parts, Sodium 1.16 parts, Magnesium 0.76 parts, Ammonium 1.02 parts, Hydrogen 0.05 parts, Salicylic Acid 2.00 parts, Chloro-Phenols 2.00 parts. One part of the above is dissolved in 115 parts of a 10% aqueous solution of Propenetriol.

Iron Jelloids No. 2 (*The Iron Jelloid Co. Ltd., Watford*). "For adults (men and women). Prevent and overcome iron-starvation. Restore health and strength to men, women and children who suffer from anæmia, exhausted nerves, lack of energy, liability to colds and slight infections, breathlessness, faintness, palpitation, heart pains, numbness, poor appetite, weak digestion, constipation, headaches, sore tongue, paleness, sallowness, falling and fading hair, brittle nails and skin blemishes." *Formula*:—Ferr. Sulph. Exsic. B.P. 19.629%, Sod. Carb. Exsic. B.P. 13.704%, Glucos. Liq. B.P. 43.266%, Saccharin B.P.C. 0.017%, Gelat. B.P. 9.833%, Vanillin B.P.C. 0.033%, Aqua Dest. 13.518%. *Dose*: 1 Jelloid.

Iron Jelloids No. 2A with Quinine (*The Iron Jelloid Co. Ltd., Watford*). "For men. Also suitable for ladies." *Formula*:—Ferr. Sulph. Exsic. B.P. 18.124%, Sod. Carb. Exsic. B.P. 12.646%, Glucos. Liq. B.P. 39.940%, Saccharin B.P.C. 0.015%, Gelat. B.P. 9.080%, Quinin. Sulph. 3.845%, Vanillin B.P.C. 0.030%, Aqua Dest. 16.320%. *Dose*: 1 Jelloid.

Iron-Ox Tablets (*Pharmaceutical Laboratories Ltd., London*). "Supertonic tablets. An invaluable tonic containing iron and manganese in a specially-prepared form." *Formula*:—Each tablet contains: Cupr. Sulph. gr. $\frac{1}{16}$, Mang. Cit. gr. $\frac{1}{32}$, Aloin gr. $\frac{1}{16}$, Caffein gr. $\frac{1}{32}$, Ext. Casc. Sagr. Sicc. gr. $\frac{1}{16}$, Oleores. Capsic. gr. $\frac{1}{16}$, Hæmoglob. gr. $\frac{1}{16}$, Ferr. Sulph. Exsic. gr. $\frac{1}{16}$, Sod. Carb. Exsic. gr. $\frac{1}{16}$.

Irvona (*The London and Colonial Export Co. Ltd., London*). "Nerve and body builder. Makes thin folk plump and weak folk strong." *Formula*:—Ferri Sulph. Exsic. B.P. gr. 1.25, Calc. Hypophos. B.P.C. gr. 1.67, Aloin B.P. gr. 0.025, Exciapiens q.s. *Dose*: 1 tablet.

Isogel Granules (*Allen & Hanburys Ltd., London*). "Indicated in all cases where intestinal action is deficient. Free from all purgatives." *Formula*:—Contains Ispagh. Test. 90.0%. *Dose*: 2 teaspoonfuls; Children, $\frac{1}{2}$ to 1 teaspoonful.

[P2] **Izal Germicidal Antiseptic** (*Newton Chambers & Co. Ltd., Thornecliffe*). Directions are given for use in mouthwashes, gargles, footbaths and washing soiled linen, and for washing wounds, etc. *Formula*:—Phenols, 40% v/v.

Jackson's Febrifuge (*George Jackson & Sons, Ltd., Manchester*). "For the cure of all kinds of fever, influenza, quinsy, bronchitis, inflammation of the lungs, etc., feverish colds, measles, chicken pox, thrush, ulcerated mouth and throat, frog and scarlatina." *Formula*:—Sucrose 4.190, Sodii Sulph. B.P. 4.190, Pot. Nit. B.P. .761, Chlorof. B.P. .13, Ammon. Chlor. B.P. .761, Ext. Tarax. B.P.C. .666, Ext. Glycyrrh. B.P. .633, Ext. Rhei B.P.C. .190, Sach. Ustum .634, Tr. Zingib. Fort. B.P. .476, Pot. Iodid. B.P. .047,

Iodum Resub. B.P. .048, Tr. Capsici B.P. .095, Camphor B.P. .023, Spiritus Rectif. B.P. 1.369, Ol. Anisi. B.P. .019, Ol. Caryoph. B.P. .039, Aqua ad to 100.000. *Dose:* Children, $\frac{1}{2}$ to 2 teaspoonfuls; Adults, $\frac{1}{2}$ to 1 tablespoonful.

Jenner's (Dr.) Absorbent Lozenge Digestive Tablets (*Savory & Moore Ltd., London*). "The well-proved remedy for indigestion, heartburn, flatulence and acidity." *Formula:*—Each tablet contains "Calsorbent" brand Calc. Carb. 1.3 Gm., Ol. Caryophylli 0.004 Gm. *Dose:* 1 or 2 tablets.

Jests (Jests Ltd., Slough). "To relieve or prevent sour stomach, heartburn, flatulence, gas and distress after eating." *Formula:*—calc. carb. 32%, mag. trisil. 12%, sweetening 54%, flavouring q.s. *Dose:* 1 to 4 tablets.

Jif Neuralgia Powders (*John Morgan Jones & Co. Agents: May Roberts & Co. Ltd., London*). "Quinsy promptly arrested, however severe." *Formula:*—Active ingredients: Acetphenetidin 15%, Acid. Acetylsalicylic 60%.

John Bell Brand Tin-Ox Tablets (*John Bell, Hills, and Lucas Ltd., London*). "The modern remedy for boils, pimples, acne, carbuncles, abscesses, etc." *Formula:*—Tin-Ox tablets contain 10 per cent. and 15 per cent. respectively of physiologically pure tin and stannic oxide together with excipient. *Dose:* 2 tablets.

Johnson's (Mrs.) American Soothing Syrup (*Barclay & Sons Ltd., London*). "For infants' teething." *Formula:*—Sod. Chlorid. 6.6%, Tinct. Tolu 2.5%.

Jordan's Brand Gin Pills (*The Jordan Medicine Co., London*). "A safe and certain remedy for backache, rheumatism, lumbago and all disorders of the kidneys." *Formula:*—Ext. Uvæ Ursi 12.698, Ol. Junip. Comm. 1.333, Ext. Buchu 3.175, Ext. Cubeb. 3.000, Ext. Gentian. 8.333, P. Fol. Buchu 11.500, Aloin 0.334, P. Fol. Uvæ Ursi 36.000, Potass. Nit. 12.698, Syrupus 10.929.

Juno-Junipah (*Thomas Marns Ltd., London*). "Mineral spring salts. Especially recommended for slimming, backache, rheumatism, lumbago, and all conditions brought about by constipation quickly disappear after taking." *Formula:*—Sod. Sulph. Exsicc. 81.4%, Sod. Chlorid. 0.80%, Sod. Phosph. Exsicc. 0.75%, Sod. Bicarb. 10.98%, Ol. Junip. 0.08%. *Dose:* 1 teaspoonful or less in water.

Juvigold Brand Gland Tonic (*The Middlesex Laboratory of Glandular Research Ltd., London*). Liquid Form. "An intensely active pick-me-up tonic for 'Invisible Lack of Health,' general debility and sluggishness of the ductless glands." *Formula:*—Colloidal Sol. Platinum 0.001%, Colloidal Sol. Palladium 0.001%, Colloidal Sol. Aurum 0.001%, Cerebrin Substance 0.125%, Cardin Substance 0.125%, Myelin Substance 0.125%, Lung Substance 0.125%, Glucose Liq. 18.5%, Lævulose 1.05%, Ferri Phos. 0.425%, Calci Lactophos. 0.425%, Trichlormethane 0.001%, Iodum et Pot. Iod. a.a. 0.0015%, Ol. Anisi. Juniper. Limonis. Menth. Pip. a.a. 0.0004%, Tinct. Capsici Fort. 0.001%. *Dose:* 10 to 16 drops.

K.12 Regulators (*The manufacturer's name is not given on the container*). "For chronic constipation. They will not gripe and are good for both old and young." *Formula:*—Phenolphthalein gr. 1 $\frac{1}{2}$.

Kaldrox Brand Adsorbent Compound (*John Wyeth & Bro., London*). "An activated fluid adsorbent." *Formula:*—Kaolinum 20%, Aluminii Hydroxidi 2.5%, Aromatici 0.008%, Aqua Destillata q.s. *Dose:* 1 tablespoonful in water.

Kalzana Brand Calcium Sodium Lactate Tablets (*Therapeutic Products Ltd., Greenford*). "Restores the vitality of all body cells. Prevents and conquers ill-health due to calcium deficiency." *Formula:*—Calcii Lactas 18.5%, Sodii Lactas 18.5%, Saccharum Purificatum 37.0%, Saccharum Lactis 10.0%, Talcum 4.500%, Acidum Tartaricum .485%, Ol. Mentha Piperitæ .010%, Menthol .005%, Amylum 11.0%. *Dose:* 2 or 3 tablets; Children, 1 or 2 tablets; Babies, $\frac{1}{2}$ to 1 tablet.

Kalzana Brand Powder (*Therapeutic Products Ltd., Greenford*). "Restores the vitality of all body cells. Prevents and conquers ill-health due to calcium deficiency. Increases power to resist disease. Improves the condition of blood and skin." *Formula:*—Calcii Lactas 25%, Sodii Lactas 25%, Saccharum Pur. 50%. *Dose:* 1 teaspoonful.

Kaputine (*General Kaputine Syndicate Ltd., Chadderton*). "Headache and neuralgia cure. It has no injurious effects upon the heart." *Formula:*—Acid Acetylsalicyl. 7 grs., Bole Armen. q.s.

Karmoid Brand Tablets (*International Laboratories Ltd., London*). "An efficacious laxative tonic for the head, stomach and bowels." *Formula*:—Phenolphthalein 2 grs. *Dose*: 1 to 3 tablets.

Karsodrine Inhaler (*E. Griffiths Hughes Ltd., Manchester*). "Instantly relieves nasal congestion, head colds, nasal catarrh, asthma, hay fever, sinusitis, etc." *Formula*:—Each tube contains: Amphetamine 0.330 grm., Cineole 0.093 grm., Ol. Citronell 0.026 grm., Methyl Salicyl. 0.023 grm., Ol. Cedri 0.005 grm., Menthol 0.049 grm., Ol. Eucalypt. 0.089 grm.

Karsote Inhalant (*E. Griffiths Hughes Ltd., Manchester*). "Affords immediate relief in cases of common colds, influenza colds, whooping cough, nasal catarrh, bronchitis, asthma, hay-fever." *Formula*:—Contains in 1000 parts of a spirituous solution the following: Ol. Absinth. 0.5, Acid Cinnam. 1.0, Benzyl Acet. 0.5, Benzyl Alc. 5.0, Borneol 0.5, Ol. Cass. 7.5, Ol. Cedri 2.5, Cinnam. Aldehyde 7.5, Camph. 35.0, Cinnam. Alcohol 1.0, Ol. Cinnam. 0.5, Cineole 85.0, Ol. Citronell 25.0, Ol. Eucalypt 95.0, Geraniol 2.5, Ol. Gram Citrat. 2.0, Limonene 2.0, Linalol 0.5, Menthyl 35.0, Methyl Salicyl. 75.0, Phellandrene 12.0, Thym. 2.5.

Kasbah Kidney Cure (*Potter & Clarke, London*). "A positive cure for backache, gravel and diseases of the kidneys and bladder." *Formula*:—Equisetum 15%, Clivers 15%, Liquorice 15%, Triticum 20%, Buchu 15%, Senna Leaf 10%, Uva Ursi 10%.

Kaye's Worsdell's Vegetable Restorative Pills (*John Kaye, Esq., London*). "For indigestion, biliousness, constipation, running sores, ulcers, abscesses. To open obstructions, improve digestion, purify the blood, produce free circulation and establish the health." The composition of this preparation was not disclosed.

Kaylene Saline (*Kaylene Ltd., London*). "A preparation combining the valuable adsorptive powers of Kaylene with those of saline laxatives." *Formula*:—Kaylene (Colloidal Kaolin) 60.579%, Sodii Bicarb. 18.182%, Sodii Sulphas Exsicc. 6.06%, P. Acid. Tartaric 12.121%, P. Acid. Citric. 3.030%, Saccharin 0.028%.

Kaylene-Ol. (*Kaylene Ltd., London*). "Kaylene Brand of colloidal kaolin with highly viscous liquid paraffin. A palatable emulsion of Kaylene and highly viscous liquid paraffin for the treatment of conditions due to toxæmia arising from intestinal stasis or any kindred causes." *Formula in accordance with the Paraffin Emulsion (Reduction of Liquid Paraffin) Order 1941*:—Kaylene (Colloidal Kaolin) 7.50%, Paraffinum Liquidum 25.00%, Acidum Benzoicum 0.08%, Aromatici 0.04%, Aqua Dest. 66.80%, Saccharin 0.01%, Gum Tragacanth 0.22%, Spirit Chloroform 0.35%. *Dose*: 1 dessertspoonful; Children, 1 to 2 teaspoonfuls.

Kaylene-Ol with Phenolphthalein (*Kaylene Ltd., London*). "A palatable emulsion of Kaylene and highly viscous liquid paraffin for the treatment of conditions due to toxæmia arising from intestinal stasis and kindred causes." *Formula in accordance with the Paraffin Emulsion (Reduction of Liquid Paraffin) Order 1941*:—Kaylene (Colloidal Kaolin) 7.50%, Paraffinum Liquidum 25.00%, Acidum Benzoicum 0.08%, Aromatici 0.04%, Aqua Dest. 66.30%, Saccharin 0.01%, Gum Tragacanth 0.22%, Spirit Chloroform 0.35%, Phenolphthalein 0.5%. *Dose*: 1 dessertspoonful; Children, 1 to 2 teaspoonfuls.

Kay's Linseed Compound (*Kay Bros. Ltd., Stockport*). "For coughs and colds. Relieves, soothes, heals and restores." *Formula*:—Ol. Anis. 0.15, Seneg. 0.2, Scill. 5, Linum 5, Mel Depur. 5, Ipecac. 0.3, Chlorof. 3, Æther 0.2, Syr. Tolu. ad 100. *Dose*: From 5 drops to a teaspoonful according to age.

Keating's Pectoral Cough Lozenges (*Thomas Keating Ltd., London*). "Greatly assist in removing the oppression and difficulty of breathing attendant upon asthmatic complaints by promoting that gentle expectoration which affords relief in coughs and other affections of the chest and lungs." *Formula*:—Ext. Glycyrrhizæ 18.0%, Aqua destil 5.6%, Ext. Marubii 1.8%, Sacchar. alb. 68.0%, Gum Acacia 6.6%.

Kephaldol Brand (*Kephaldol Laboratories Ltd., London*). "For rheumatism, arthritis, lumbago, sciatica, neuritis, neuralgia, headaches, menstruation pains, all severe nerve pains, hysteria, nervous irritability, sleeplessness, feverishness, malaria, influenza, feverish colds, hay fever." *Formula*:—Acetphenetidin 2.5 gr., Sodium Salicylate 1.75 gr., Quinine 0.45 gr., Caffeine 0.25 gr., Salicylic Acid 0.15 gr., Citric Acid 0.25 gr., Amylum q.s. *Dose*: 1 or 2 tablets.

Kepler Cod Liver Oil with Malt Extract (*Burroughs Wellcome & Co., London*). "An ideal dietary supplement containing fats, carbohydrates and

vitamins necessary for perfect physical development and resistance to disease." *Formula*:—Containing 23% *v/v* of Cod Liver Oil. *Dose*: 1 teaspoonful to 2 dessertspoonfuls.

Kerol Medical Capsules (*H. R. Napp Ltd., London*). "Intestinal 3 minims." *Formula*:—Each capsule contains 1.3 minim of Phenols.

Kerol Medical Capsules (*H. R. Napp Ltd., London*). "Gastric 3 minims." *Formula*:—Each capsule contains 1.3 minim of Phenols.

Kest Brand Compound Epsom Salts Tablets (*Kest Ltd., London*). "Keep the bloodstream pure, keep the bowels free by taking Kest Brand Tablets—a natural medicine in an easy-to-take form." *Formula*:—Each tablet contains 4½ gr. Mag. Sulph., ½ gr. Phenolphthalein." *Dose*: 1 or 2 tablets.

Keswick's Pure Vegetable Charcoal (*J. B. Keswick & Co., Wigton*). "Indigestion, flatulency, biliousness, pains in the stomach, headaches, neuralgia and neuritis." *Dose*: 1 to 5 tablets.

Keswick's Pure Vegetable Charco-Lax (Tablets) (*J. B. Keswick & Co., Wigton*). "An effective bowel remedy for young and old." *Formula*:—R Pil. Rhei Co. 4.00%, P. Saponis .50%, Aloin .50%, Ol. Racini q.s., Pu. Carbolignin add 100.00%. *Dose*: 1, 2, or 3 tablets; Children, ½ to 2 tablets.

Ki-uma Brand Ointment (*Ki-uma Ltd., Bath*). Salicylic Ester Dihydroxethane 15%, Cetaceum 3.5%, Ol. Eucalypti Glob. 1.5%, Ol. Bassiæ Parkii 80%, Rad. Anthusii q.s.

Ki-uma Brand Plasters (*Ki-uma Ltd., Bath*). Bassiæ Parkii 37%, Kaolin (Colloidal) 37%, Paraffin Moll. Flav. 6%, Glycerin 13%, Salicylic Ester Dihydroxethane 4%, Pine Oils 2.4%, Eucalyptus 0.6%.

Kompo (Dr. White's) (*J. F. White & Co. Ltd., Leeds*). "A valuable remedy for colds, influenza, diarrhoea, pains in the stomach and bowels, disordered stomach, cold feet, cold sweats, bad circulation of the blood, sore throat, quinsy, etc." *Formula*:—Kutch 5.9%, Ol. Caryoph. .065%, Ol. Cass. .09%, Tinct. Capsic. 7.29%, Aqua q.s. *Dose*: 1 to 2 teaspoonfuls.

Koray Brand Tablets (*Koray Ltd., London*). "For headache, neuralgia, rheumatism, lumbago, sciatica, neurasthenic pains, earache, faceache, colds, influenza, women's pains and resultant depression, feverishness, sore throat, sleeplessness, malaria." *Formula*:—Acid Acetyl Salicylic 7.5 grs., Exc. 0.5 grs. (approx.), Sucrose 0.25 grs. (approx.), Sodium Tetra-Bromo-Fluoresceine q.s.

Kotalko (*John Hart (Britain) Ltd.*). "For hair growth. For incipient baldness. Kotalko is not coloured, dyed nor perfumed artificially." *Formula*:—Oleum pici 7.5%, Ol. Camph. rect. 9.00%, Resorcinol 0.63%, Oleoresina Capsici 0.03%.

Kruschen Salts (*E. Griffiths Hughes Ltd., Manchester*). "A perfect diuretic aperient, suitable for persons suffering the pains of rheumatism, gout, lumbago, etc., and for habitual constipation with inactive liver." *Formula*:—Kruschen salts is a therapeutically-balanced preparation giving the following ionic ratios in solution:—Magnesium (Mg.) 7.895, Potassium (K) 2.992, Sodium (Na) 4.583, Chloride (Cl) 6.541, Sulphate (SO₄) 35.568, Citrate (C₆H₅OH(COO)₃) 1.350, this bottle containing Sodii Sulphas (Anhyd.) 1.16 G., Sodii Chloridum 5.80 G., Potassii Chloridum 0.58 G., Potassii Sulphas 3.19 G., Acidum Citricum 0.87 G., Magnesii Sulphas quantum sufficit ad 58 G. approx.

Kutnow's Powder (*Kutnow & Co., Leeds*). "A pleasant and effective aperient." *Formula*:—Soda Tartarat. 41.14%, Sodii Bicarbonat. 25.40%, Acid Tartaric 23.15%, Sodii Sulphat. 10.28%, Saccharin 0.03%.

Lactagol (*Lactagol Ltd., E. T. Pearson & Co., Mitcham*). "A food for increasing and enriching the supply of milk when taken by nursing mothers. A pleasantly flavoured powdered extract of cotton seed with iron, calcium and phosphorus in suitable proportions." *Formula*:—Protein 62%, Calcium 1.8%, Iron 0.12%, Phosphorus 1.1%, Chocolate to make up to 100%.

Lacteol Brand Tablets (*Dr. Boucard*) (*Wilcox, Jozeau & Co. Ltd., London*). "Indication, enteritis acute or chronic, diarrhoea, foetid motions, colitis, gastro-enteritis in infants, skin affections following intestinal upset, etc." *Formula*:—Each tablet contains: 2,480,000 Lactic Bacilli (½ bac. lactis communis, ½ bac. bulgaricus) excipient q.s. to 0.55 g. *Dose*: Adults, 3 tablets; Children, 1 to 2 tablets.

Lacto-Calamine (*Crookes Laboratories, London*). *Formula*:—Calamine B.P.C. 11.5%, Liq. Hamam. B.P.C. 10%, Sorbitol 3%.

Lactopeptine Elixir (*Products (Beechams) Ltd., St. Helens*). "Invaluable in cases of indigestion, dyspepsia, flatulence, and other forms of stomach and digestive troubles." *Formula*:—Pepsinum 5.00, Acidum Hydrochloricum 0.20, Acidum Lacticum 0.10, Syrupus 19.85, Alcohol 16.34, Olea Essentia (Aurant.,

Coriand., Cinnam) 0·05, Aqua ad 100·00 (parts by weight). *Dose*: 2 to 4 teaspoonfuls.

Lactopeptine Brand Powder (*Products (Beechams) Ltd., St. Helens*). "Invaluable in cases of indigestion, dyspepsia, flatulence, and other forms of stomach and digestive troubles." *Formula*:—Acidum Lacticum 1·45%, Pepsinum (10,000) 5·25%, Pancreatinum (XXX) 3·00%, Diastasum 5·90%, Betainæ Hydrochloridum 1·00%, Lactosum 83·40%.

Lactopeptine Tablets (*Products (Beechams) Ltd., St. Helens*). "For indigestion, dyspepsia, flatulence, heartburn, acidity." *Formula*:—Pepsinum (10,000) 5·25%, Pancreatinum (XXX) 3·00%, Diastasum 5·90%, Acidum Lacticum 1·00%, Acidum Citricum 0·3%, Saccharinum Solubile 0·26%, Essentia Ananassæ Sativæ 0·25%, Betainæ Hydrochloridum 1·00%, Creta Gallica Purificata 5·00%, Lactosum 77·51%. *Dose*: 2 to 4 tablets.

Lalkala (*Lundy, Wilson & Co. Ltd., Maidstone*). "Immediately relieves asthma, bronchial catarrh, hay fever, etc." *Formula*:—Each absorbent paper base is impregnated with Potass. Nitras. Pulv. grains 12·15, Ext. Stramonii Fol. grains 7·45, Tinct. Benzoin Co. grains 6·48.

Lalkala Cigarettes (*Lundy, Wilson & Co. Ltd., Maidstone*). "Immediately relieves asthma, bronchial catarrh, hay fever, etc." *Formula*:—Absorbent paper base of each cigarette is impregnated with Potass. Nitras Pulv. grains 1·42, Ext. Stramonii Fol. grains 2·08.

Lanalol (*Lanalol Ltd., London*). No. 1 with oil. "The natural hair food. Being a purely natural and organic hair food, containing no injurious chemicals, Lanalol is readily and completely absorbed by the scalp, as well as by every single hair from root to tip. Lanalol can't help making the hair grow because it is made from hair-growing glands." The composition of this preparation was not disclosed.

Langdale's Compressed Cinnamon Tablets (*E. F. Langdale, London*). "An effective and reliable remedy for colds and influenza." *Formula*:—Ol. Cinnamoni Cort. 2·00, Tr. Ipecacuanhæ ·50, Tr. Scillæ ·50, Tr. Senegæ ·50, Sugar 96·00, Gum Tragacanth. and Veg. Color ·50. *Dose*: 1 tablet.

Langdale's Compressed Self-Aid Peppermint Tablets (*E. F. Langdale, London*). "An effective and reliable remedy and cure for nausea, heartburn, griping, flatulence, indigestion, hysteria, lowness of spirits and depression." *Formula*:—Each tablet contains ten drops of Langdale's Concentrated Self-Aid Essence of Peppermint.

Langdale's Concentrated Medicinal Essence of Cinnamon (*E. F. Langdale, London*). "For coughs, colds, etc., its warming aromatic qualities gives relief in any incredibly short space of time." *Formula*:—Ol. Cinnamoni Cort. 6·40%, Tinct. Senegæ ·50, Tinct. Scillæ ·75, Tinct. Ipecacuanhæ ·75, Alcohol 60 o/p 57·00, Aq. Distill. 32·00, Liq. Veg. Col. 2·60. *Dose*: Adults, half a teaspoonful; Children over 5 years, 10 drops.

Langdale's Concentrated Medicinal Essence of Peppermint (*E. F. Langdale, London*). "For use in the relief of nausea, griping, flatulence, indigestion, etc." *Formula*:—Ol. Menthæ Pip. 7%, Alcohol 70%, Aq. Destillata 23%. *Dose*: Adults, 10 drops; Children age 7 to 14 years, 6 drops.

Laxobac Laxative Chocolate (*E. Griffiths Hughes Ltd., Manchester*). *Formula*:—Phenolphthal 10%, Theobrom. Præp. 30·6%, Sacrose 19·8%, Amylum 22·5%, Ol. Theobrom. 17·1%. *Dose*: 2 sections; Children, $\frac{1}{2}$ to 2 sections according to age.

Le Roi Brand Albanian Salve (*Anthony (Chemist) Ltd., Cardiff*). "Surpasses everything for sores, wounds, eruptions, etc. Soothing, healing, antiseptic." *Formula*:—Paraff. Dur. 5·6338%, Colophonium Flav. Pur. 7·5117%, Zinci Oxid. B.P. 4·2275%, Phenol Liq. B.P. '14 1·4084%, Ol. Olivæ Opt. 4·2275%, Ol. Terebinth. Rect. 5·6338%, Paraff. Moll. Flav. ad 100%.

[P] **Lewis's Drops** (*Morgan & George, Ystrad, Rhondda*). "A certain cure for inflammation, pleurisy, quinsy, diarrhoea, sore throats, bronchitis, asthma, bad coughs, pains of any kind and all affections of the chest and lungs." *Formula*:—Tr. Opii (B.P. '98) 8·0%, Spts. Æther Nitrosi 3·2%, Camphor 3·28%, Spts. Vini Rect. 63·32%, Aquam ad 100. *Dose*: 1 year and upwards 1 drop, 2 years 2 drops, 3 years 3 drops, etc.; Adults, 30 to 40 drops in water 3 times daily.

Licoricine (*Hall, Forster & Co., Newcastle-on-Tyne*). "For coughs and colds." *Formula*:—Sucrosum 18·48, Glycyrrhiza 2·73, Acetum Scillæ 4·17, Spiritus Rectificatus 0·28, Chloroformum 0·36, Camphora 0·036, Acidum Benzoicum 0·054, Oleum Anisi 0·067, Acacia 2·12, Chondrus 0·68, Saccharum

Ustum 0.064, Aqua 70.059. *Dose:* Children under 4 years, 10 to 20 drops; Adults, 2 teaspoonfuls.

Limestone Brand Phosphate Compound (*International Chemical Co. Ltd., London*). "A valuable aid to perfect health." *Formula:*—7.64% Sodium Phosphate in a flavoured effervescent base.

Liquifruta (*The Liquifruta Laboratories, London*). "Herbal only; for congestion, whooping cough and other coughs. Children's coughs, nasal catarrh, bronchitis, asthma, congestion, cough, croup, influenza, pneumonia, pleurisy." *Formula:*—Ol. allii 0.0052, Ol. menth. pip. 0.078, Ol. anisi 0.041, sem. lini 2.0, succ. glycyrrh. 1.75, sacch. ust. 0.125, sacrosum 1.875, chond. 0.625, cetrar. 0.875, anthem. 0.375, aq. ad 100. *Dose:* $\frac{1}{2}$ to 2 teaspoonfuls.

Liquor Carbonis Detergens (*Wright, Layman & Umney, London*). "For eczema, psoriasis, pruritis, alopecia." *Formula:*—Ext. Quillaia 2.58, Pix Carbonis B.P.C. 20.000, Solvents ad 100.

Listerine Brand Antiseptic (*Lambert Pharmacal Co., London*). "An efficient non-poisonous germicide, the safe antiseptic with the pleasant taste. Can be used freely as a lotion, gargle or spray." *Formula:*—It is composed of thymol, eucalyptol, methyl salicylate, menthol, baptisia, benzoic acid of each one part; boric acid twenty-nine parts, ethyl alcohol two hundred and fifty parts, water to make one thousand parts.

Little Movies (*The manufacturer's name is not given on the container*). "A new and pleasant laxative preparation for the use of children and adults." *Formula:*—Hydrarg. Subchlor. $\frac{1}{2}$ gr., Excipient q.s.f.t. Chocolate coated. *Dose:* 1 or 2 tablets.

Lixen Elixir (*Allen & Hanburys Ltd., London*). "The good-natured laxative." *Formula:*—Ext. Sennae Legum 9.00, Syrup 47.80, Glycerine 6.15, Spt. Rect. 12.80, Flavouring q.s., Aq. Distill. ad 100.00. *Dose:* Adults, $\frac{1}{2}$ to 1 teaspoonful; Children, 15 drops, more or less according to age.

Lombio (*The Lombio Co., Watford*). "The certain cure for piles, eczema, burns, scalds, etc." *Formula:*—Plumb. Monox. 16.778%, Paraff. Dur. 8.054%, Paraff. Moll. Flav. 75.168%.

Lombio Suppositories (*The Lombio Co., Watford*). "Invaluable to sufferers from internal piles." *Formula:*—Plumb. Monox. 16%, Paraff. Moll. Flav. 42%, Cetaceum 42%.

Luma Brand Anti-Rheumatic Compound (*Luma Products Ltd., Manchester*). "Wintergreen, iodine and capsicine anti-rheumatic compound. Wintergreen and iodine applied in this form are rapidly absorbed through the pores of the skin, giving rapid relief from pain." *Formula:*—Methyl Salicylate (Wintergreen Oil) 6.0%, Sodium Iodide 0.4%, Oleo Resina Capsici 0.01%, Fluorescin 0.5%, Sodium Carbonate to 100.0%.

Lusty Brand Garlic Tablets (*Lusty's Natural Products Co., Westcliff-on-Sea*). "A natural purifier for the whole system. Garlic is invaluable for catarrh, chest complaints, eczema, fevers, rheumatism, impure blood, ulcers, gastritis, high blood pressure, and wherever an internal antiseptic is required." *Formula:*—Each guaranteed to contain the equivalent to 20 minims Succus Allii. *Dose:* 2 tablets.

Lyc Yeastlets (*The Standard Yeast Co. Ltd., London*). "Pure yeast culture tablets. Strengthens your vitality. Improve complexion. Aid digestion and tone up system. Purify the blood." *Formula:*—*B* Saccharomyces. *Dose:* 2 tablets.

[P1] **Lysolats** (*Solidol Chemical Ltd., London*). "Lysol tablets. The safe way to use the world's finest antiseptic and disinfectant." *Formula:*—Sapo-Stearo-Palmitic 0.1, Para-Meta-Ortho Cresols ad 1.0.

Mac Brand Antiseptic Throat Sweets (*Macleans Ltd., London*). "For sore throat, tonsillitis, catarrh, coughs, colds, etc." *Formula:*—Amyl-Oxy-Toluen 0.016, Camph. 0.006, Menthol. 0.252, Eucalyp. 0.06, Tinct. Tolu. 0.043, Ol. Menth. Pip. 0.043, Ol. Anis. 0.020, Ol. Cass. 0.025, Ol. Caryoph. 0.013, Acid. Tart. 0.347, Sugar base to 100.

Maclean Brand Stomach Powder (*Macleans Ltd., London*). "For indigestion, dyspepsia, flatulence, gastritis and all forms of stomach disorder." *Formula:*—A unique combination in equal proportions by weight of Calcium Carbonate and Heavy Magnesium Carbonate produced in accordance with British Patents No. 532300 and No. 539157, 72.73%; a special Sodium Bicarbonate produced in accordance with British Patent No. 532301, 18.18%, Bismuth Carbonate 9.09%. *Dose:* 1 teaspoonful.

Maclean Brand Stomach Tablets (*Macleans Ltd., London*). "Should be used for indigestion, flatulence, heartburn, gastritis, and all forms of stomach disorder." *Formula:*—A unique combination in equal proportions by weight

of Calcium Carbonate and Heavy Magnesium Carbonate produced in accordance with British Patent No. 532300, 72.73%; a special Sodium Bicarbonate produced in accordance with British Patent No. 532301, 18.18%; Bismuth Carbonate 9.09%. *Dose:* 2 to 4 tablets.

McClure Brand Ephedrine Nasal Catarrh Specific (*McClure, Young & Co. Ltd., London*). "An immediate relief from the discomfort of colds, catarrh, asthma, hay-fever and all affections of the nasal mucous membrane." *Formula:*—Ephedrine 0.99%, Camphor 2%, Menthol 2%, Aromatics 2%, Liquid Paraffin 93.01%.

Magsorbent Glucose Granules (*Kaylene Ltd., London*). "Indications: Acidosis, ketosis, cyclical vomiting, insulin reaction, also train, car, air and sea sickness." *Formula:*—Glucose B.P. 73.20%, Magsorbent (Magnesium Trisilicate) 24.05%, Ol. Menth. Pip. 0.65%, Excipient 2.10%. *Dose:* 1 or 2 teaspoonfuls.

Magsorbent Glucose Tablets (*Kaylene Ltd., London*). "A pleasant carminative combination of pure medicinal Glucose (Dextrose) and Magsorbent, flavoured with finest oil of Peppermint." *Formula:*—Glucose B.P. 73.68%, Magsorbent (Magnesium Trisilicate) 21.57%, Ol. Menth. Pip. 0.72%, Excipient 4.03%. *Dose:* 1 to 3 tablets.

Man Zan Brand Pile Remedy (*E. C. De Witt & Co., London*). "For blind, bleeding, itching and ulcerated piles." *Formula:*—Eucalyptus .28%, Phenol 1.78%, Camphor .06%, Thymol .01%, Witch Hazel Ext. 1.10%, Paraff. Molle q.s. to 100%.

Marienbad Anti-Obesity Tablets (*Sanarol Brand Products, London*). "A pleasant and safe home treatment." *Formula:*—Ext. Cascara Sag. Sicc. 0.49 grains, Ext. Aloes Sicc. 0.49 grains, P. Podophyllum 0.15 grains, P. Rhiz Rhei 0.98 grains, Sal. Marienbad Art. 0.23 grains. *Dose:* 1 or 2 tablets.

Marmola Brand Antifat Tablets (*Agent: 86 Clerkenwell Rd., London*). "For the treatment of obesity." *Formula:*—Ext. Fucus Vesic. gr. 2½, Ext. Phytolacca, gr. ½, Ext. Cascara Sag. gr. ½, Calc. Carb. prec. gr. 3, Phenolphthalein gr. ½, Oleoresin Zingiber, min. 16/100, Mixt. aa Ol. Anisi, Ol. Sassafras and Methyl Salicylas. min. ½, Sucrose q.s. *Dose:* 1 tablet.

Mason's Herb or Botanic Beer (*Newball & Mason, Nottingham*). "This preparation containing Yarrow, Dandelion, Comfrey and Horehound makes an excellent non-intoxicating beverage." No further indication of the composition was given.

Matthews Brand Fullers Earth Cream (*Rouse Bros. Ltd., London*). "For all forms of skin inflammation. Healing, soothing and softening." *Formula:*—Ceresine 7.386, Adeps Lanæ Anhyd. 3.409, Paraffin Molle 77.642, Phenyl Hydrate 0.07, Calc. Carb. 2.982, Zinc. Oxide 2.982, Fullers Earth 5.398, Musk Zylol 0.019, Oil of Cloves 0.026, Oil of Geranium 0.049, Oil of Citronel 0.013, Terpeneol 0.024.

Menopax Brand Tablets (*Clinical Products Ltd., Richmond*). *Formula:*—Stilboestrol Dipropionate .025 mgm., Ovarian Substance .25 gr., Menthol .50 mgm., Valeriana 1.00 gr., Caffeina et Sod. Sal. .15 gr., Calcium Lactate 1.50 gr., Excipient ad 3.50 gr. *Dose:* 1 to 2 tablets.

Medicated Chocolate Cakes (*R. Gibson & Sons, Manchester*). *Formula:*—Calomel gr. 2, Jalap gr. 2, and ½ Santonine. *Dose:* ½ to 1 cake for children from 2 to 4 years; after that age 1 to 1½ cakes.

Medilax Brand Laxative Pellets (*Savory & Moore Ltd., London*). "A rational specific for the treatment of constipation and its allied troubles. Non-habit forming, non-gripping. An effective aperient for general and regular use." *Formula:*—Each pellet contains Podoph. Res. gr. ½, Saponis gr. ¼, Aloe gr. ½, Scammon. Res. gr. ½, Ext. Colocynth. Co. gr. ½, Phenolphthal. gr. ⅓, Oleores. Zingib. gr. ⅓. *Dose:* 1 to 3 pellets.

Meggeson Brand Bismuth Dyspepsia Tablets (*Meggeson & Co. Ltd., London*). "For indigestion, flatulence and acidity." *Formula:*—Bis. Carb. 7.69%, Sodii Bicarb. 23.07%, Chlorol. 7.23%, Lavender lozenge base to make 100%. *Dose:* 1 to 2 tablets.

Meggezones (*Meggeson & Co. Ltd., London*). "For all throat affections. The foremost preparation for relieving the most acute catarrhal conditions. Hygienic, antiseptic, germicidal, soothing." *Formula:*—Menthol 0.78, Ol. Menth. Pip. 0.33, Chlorof. 1.30, Benzoin 0.28, Liquorice Pastille Basis ad 100.

Melba Iodised Throat Tablets (*The manufacturer's name is not given on the container*). "For sore throat, huskiness, ulceration, tonsillitis, etc." *Formula:*—Tinct. Iodine m. ⅓, Acid Carbolic m. ⅓, Menthol gr. ⅓, Acid Citric gr. ⅓, Ol. Gautheria m. ⅓, Sugar q.s.

Mendaco Brand (*Knox Laboratories Ltd., London*). "Prescription to end cough, choking, wheezing, gasping, shortness of breath, nervousness, sleepless nights, indigestion, headaches, and loss of vitality caused by asthma, bronchitis, hay fever." *Formula*:—Potassium Iodide 2 grs., Lobelia Ext. (Penick) $\frac{1}{2}$ gr. (\equiv alkaloids, 0.092%), Euphorbia Pil 1-5 $\frac{1}{2}$ gr., Chionanthin. $\frac{1}{2}$ gr., Excip. q.s. *Dose*: 2 tablets.

Mensal Pills (*The Mackenzie Medicine Co., London*). "Relieve painful periods, prevent sick headache, depression of spirits, nausea, languor, sallow complexion and disorders peculiar to ladies." *Formula*:—Purified Soc. Aloes 1 gr., Iron Sulph. Exsic. $\frac{1}{2}$ gr., Powd. Jam Gin. $\frac{1}{2}$ gr., Ext. Black Hellebore $\frac{1}{2}$ gr., Gum Myrrh $\frac{1}{2}$ gr., Castille Soap $\frac{1}{2}$ gr., Powd. Canella $\frac{1}{2}$ gr. *Dose*: 1 or 2 pills.

Mentex (*Foster-McClellan Co., London*). "Antiseptic, healing, analgesic, inhalant. An iodine vaporising saline for nasal catarrh, sore throat, neuralgia, painful joints." *Formula*:—Ol. Camph. Ess. 3.261, Ol. Pini Abiet. 2.447, Ol. Pini Pumil. 0.394, Methyl Salicyl. 5.369, Ol. Eucalypt. 0.415, Iodine 0.076, Camphor 1.812, Menthol 0.950, Ac. Benzoic. 0.475, Paraff. Dur. 7.839, Adeps. Lanæ Anh. 15.202, Paraff. Moll. ad 100.000.

Mentholated Bronchial Lozenges (*Parke Davis & Co., London*). *Formula*:—Each lozenge contains: P. Ext. Glycyrrh 2 gr., F.E. Coltsfoot $\frac{1}{10}$ min., Oleores Cubeb $\frac{1}{10}$ min., Balsam Tolu. $\frac{1}{10}$ gr., F.E. Capsicum $\frac{1}{10}$ min., Oil Anise $\frac{1}{2}$ min., Menthol q.s. "Owing to an acute shortage of Glycyrrhiza, we have been forced to reduce the quantity of Ext. Glycyrrhiza in each lozenge to $\frac{1}{2}$ grain."

Mentholatum Brand Antiseptic Nasal Liquid (*The Mentholatum Co., Ltd., Slough*). "For catarrh, chronic colds in the head, and all kinds of nose, throat and sinus troubles caused by colds and catarrh." *Formula*:—Menthol .5, Camphor .25, Ol. Eucalypt. .03, Ol. Pini Pum. .03, Ol. Gautherie .03, Paraff. Liq. to 100.

Mentholatum Brand Balm (*Mentholatum Co., Ltd., Slough*). "For colds in the head, catarrh, influenza, hay fever, chilblains, chapped skin, cuts, burns, skin irritation, etc." *Formula*:—Ac. Boric Pulv. 3, Menthol. 0.5, Flor. Camph. 3, Ol. Eucalypt. 0.2, Ol. Pini Pum. 0.2, Ol. Gautherie 0.2, Paraff. Moll. Flav. 30.

Metanium Brand Ointment (*Meta-Titanium Laboratories Ltd., London*). "For dry irritation of the skin, for rashes, burns (of every sort), scalds, fissures of the breasts of nursing mothers, insect bites and stings, chilblains and hæmorrhoids." *Constituents*: Approx. percentages: Titanium Tannate 0.1, Titanium Salicylate 3.0, Titanium Borate 5.0, Titanium Peroxide 5.0, Titanium Oxide 12.0, Excipient q.s.

Milk of Magnesia Brand (*Distributors: Proprietary Agencies Ltd., London*). "Antacid, laxative. A soothing application for external use. A superior mouth antacid." *Formula*:—Contains approximately 8.45% Magnesium Hydroxide (Milk of Magnesia Brand). *Dose*: Antacid, 1 or 2 teaspoonfuls; Laxative, 2 to 4 tablespoonfuls.

Milk of Magnesia Brand Tablets (*Distributors: Proprietary Agencies Ltd., London*). "To relieve indigestion and sour stomach. For a mild laxative." *Formula*:—Each tablet contains 4.8 grains Magnesium Hydroxide (Milk of Magnesia Brand). *Dose*: 1 to 4 tablets.

Mil-Par Brand Laxative (*Distributors: Proprietary Agencies Ltd., London*). "Containing Milk of Magnesia with 25% Liquid Paraffin. Reduces irritation and thus provides painless bowel movement in piles. Being gentle in action and free from any harmful or unpleasant effect, it is especially useful during pregnancy and lactation and for the aged or invalid." *Formula*:—Each fluid ounce contains 26 grains of Magnesium Hydroxide and 120 minims of Medicinal Paraffin. *Dose*: 1 or 2 tablespoonfuls.

Milton (*Milton Proprietary Ltd., London*). "Non-poisonous antiseptic." *Formula*:—Sodium Hypochlorite 1%, Sodium Chloride 16.5%, Sodium Chlorate .13%, Sodium Carbonate .05%, Sodium Sulphate .15%, Calcium Chloride .07%, Magnesium, a trace, Water 82.1%.

Milton Brand Antiseptic Ointment (*Milton Proprietary Ltd., London*). "For bad legs, bed sores, boils, chilblains, chapped skin, cold sores, sunburn, pimples, piles, bites and stings." *Formula*:—Sodium Hypochlorite .10%, Sodium Chlorate .01%, Sodium Chloride 1.65%, Magnesium Hydrate 2.00%, Paraffin 84.00%, Dichloramine T 1.00%, Calcium Carbonate 3.00%, Water to 100.0.

Minadex Brand Syrup (*Glaxo Laboratories Ltd., Greenford*). *Formula*:—Each fl. oz. contains 18,000 i.u. vitamin A and 3,000 i.u. vitamin D, Ferr. et Ammon. Cit. Vir. 13½ gr., Calc. Glycerophosph. 2 gr., Pot. Glycerophosph. ¼ gr., Sod. Glycerophosph. ½ gr., Mang. Glycerophosph. ⅓ gr., Cupr. Sulph. ⅓ gr. *Dose*: ½ to 2 teaspoonfuls.

Mistol (*Stemco Ltd., London*). "For colds, catarrh, etc." *Formula*:—Camphor 4.72%, Eucalyptol 0.630%, Menthol 0.630%, Chlorbutanol 1.008%, Sedan 4 Colouring 0.0034%, Liquid Petrolatum 97.25966%.

Mistol Drops with Ephedrine (*Stemco Ltd., London*). "Aid in the relief of head colds. To be used in acute cases under physician's directions." *Formula*:—Camphor 0.63%, Eucalyptol 0.63%, Menthol 0.63%, Ephedrine 0.57%, Green Colouring 0.0013%, Liquid Petrolatum 97.5387%.

Moffat's (Dr.) Remedy (*Dr. Moffat's Remedy Co., Cardiff*). "Hooping cough, croup and bronchitis remedy. Certified purely vegetable, free from poison and irritants." *Formula*:—Oleum Succini Rect. 35, Ol. Solvenol 4, Ol. Olivæ B.P. 25, Ol. Ref. N.W. pini 25, Ol. Eucalypti 10, Ess. Colr. 1.

M.O. Magnesia (*Musterole Fine Products Co. Ltd., Farnham*). "For sour stomach, acidity, constipation and similar conditions." *Formula*:—Active ingredients: Mag. Calcinata Levissima 4.24%, Paraffinum Liquidum 24.69%. *Dose*: 1 tablespoonful.

Monceda Fruit Cubes (*Moore Medicinal Products Ltd., Aberdeen*). "Formerly known as Neda. Nature's way with constipation." *Formula*:—Fruct. Ficis Caricæ 7-8, Fol. et Follic. Senn. 0.5, Pulpa Tamarind. 1.0, Paraffin. Liquid. 0.2. *Dose*: ½ to 1 cube.

[P2] Monsol Brand Germicide (*Monsol Ltd., London*). Directions given for use in dressings, douches, tampons, sprays, packing, etc. *Formula*:—Ol. Morrhue sulphonomum 17%, Sapo Animalis 3%, Ol. Picis (Mond) 73%, Terpinoline 2%, Aqua 5%. "Contains Phenols 30% v/v."

Monsol Brand Ointment (*The Mond Staffordshire Refining Co. Ltd.*). "For eczema, wounds, piles, bad legs, sores, scabies, ringworm, ulcers, and all skin troubles. For sprains, bruises, rheumatism, etc." *Formula*:—Adeps Lanæ 74%, Paraffinum Molle 18%, Ol. Picis (Mond) 5%, Cera Flava 3%, Isonone a trace.

Monsol Brand Pessaries (*Monsol Ltd., London*). "One to be used each night for six nights and then one every third night; unless otherwise directed by the Physician." The composition of this preparation was not disclosed.

Monsol Brand Throat Pastilles (*Monsol Ltd., London*). "For the disinfection of the throat and mouth." *Formula*:—Ol. Picis (Mond) m. 1, Ol. Anisi m. ½, Ol. Aurant. m. ½, Dextrose gr. 7, Acacia and sugar base.

Moorland Indigestion Tablets (*W. B. Cartwright Ltd., Leeds*). "The pleasant and most reliable aid to digestion." *Formula*:—Magnesii Carbonas Ponderosus 5.18%, Pepsinum 0.075%, Calcii Caronbas 40.16%, Sodii Bicarbonas 3.78%, Pancreatinum 0.05%, Bismuthi Carbonas 0.84%, Acacia 3.46%, Chloroformum 1.05%, Æther 0.52%, Sacrose 43.57%, Capsicin 0.005%, Oleum Cardamomi 0.044%, Oleum Lavandulæ 0.022%, Otto Rosæ 0.011%, Saccharinum 0.0038%, Talcum Purificatum to 100.000. *Dose*: 1 or 2 tablets.

Morse's (Dr.) Indian Root Pills (*W. H. Comstock Co. Ltd., London*). "The standard family medicine. A searching, cleansing remedy, and a positive and permanent cure for all diseases arising from constipation, impure blood, liver and kidney complaints and female ailments." *Formula*:—Aloes B.P. 38.3, Podophyllum Peltatum 23.3, Piper. Nig. 16.6, Gambogia 13.2, Jalap 6.6, Hydrarg. Subchlor. 2.0. *Dose*: 2 to 4 pills; Children, ½ to 1 pill.

Morton's Elder and Peppermint Life Drops (*Morton Mfg. Co., Motherwell*). "A powerful heart and nerve tonic and stimulant. For influenza, bronchitis, croup, pneumonia, cramp, cough, cold, rheumatism and all inflammatory diseases." *Formula*:—Tinct. Achilleæ, 25 parts, Tinct. sambuci 15 parts; Ext. asclepias 5 parts; Ol. menth. pip. 2.5 parts Tinct. capsici fort to 100 parts. *Dose*: 5 to 15 drops.

Mother Siegel's Brand Digestive Syrup (*A. J. White Ltd., London*). "A herbal medicine of unequalled value to sufferers from indigestion and other disorders of the stomach and liver." *Formula*:—Extracts of Iris 0.854, Leptandra 0.854, Stillingia 0.854, Phytolacca 0.854, Juglans 0.854, Taraxacum 0.854, Chimaphylla 0.685, Podophyllum 0.546, Gentian 0.273, Colocynth. 0.263, Cimicifuga 1.36, and Sodium Borate 3.825, Aloes 1.13, Sassafras 1.36, Capsicum 0.2, Acid. Hydrochlor. 3.19, Cane Syrup 61.2, Aq. dest. q.s. *Dose*: 15 to 20 drops.

[P1] **Mothersill's Seasick Remedy** (*Mothersill Remedy Co., London*). "Intended to relieve as well as to prevent sea-sickness, train-sickness, nausea or headaches caused by motion, climbing, etc." *Formula*:—(Pink Capsules) Chlorbutol 2 gr., Hyoscine Hydrobromide $\frac{1}{10}$ gr., Caffeine $\frac{1}{2}$ gr.; (Brown Capsules) Same as pink, plus P. Glycyrrh. Comp. U.S.P. $\frac{1}{2}$ gr. *Dose*: 1 pink and 1 brown capsule.

[P1] **M-Rex Brand Pile Ointment** (*The manufacturer's name is not given on the container*). "Helps to give ease and relieve irritation." *Formula*:—Adeps Lanæ Anhyd. 27.00%, Paraff. Mol. Flav. 25.00%, Paraf. Liq. 24.00%, Pulvis Gallæ 12.00%, Acidi Borici 2.20%, Menthol 1.13%, Liq. Hamamelidis 6.00%, Oleum Origanum 0.77%, Pulvis Opii B.P. 1.90% w/w.

Muller Nutrient (The). (*The Muller Laboratories Ltd., Manchester*). "Brand of Concentrated Nerve Food. Of great value in all cases of mental, nervous and physical exhaustion." *Formula*:—Sodii Phosph. 0.372, Magnesii Phosph. 0.744, Ferri Perphos. 0.372, Pulv. Laitum 2.172, Sucrose Alb. 4.840. One tablet 8.500 gr. *Dose*: 2 tablets.

Musterole Brand Mustard Ointment (*Musterole Fine Products Co. Ltd., Farnham*). "Try Musterole Brand Mustard Ointment for chest cold, chest cough, sore throat, tonsillitis, congestion, croup, bronchitis, 'flu (often prevents pneumonia), muscular rheumatism, lumbago, backache, pain in joints, sprains, stiff muscles, stiff neck, bruises, neuritis, neuralgia, face-ache." *Formula*:—Active ingredients Camphor (syn) 5.36%, Menthol (syn) 4.46%, Ol. Sinap. Ess. (syn) 2.68%, in a base of inert animal and mineral fats.

Mycolactine "Opocrin" Brand (*Anglo-French Drug Co. Ltd., London*). "An intestinal disinfectant and educator in all bowel complaints." *Formula* (per tablet):—Ext. Cerev. Ferment. 0.10 Gm., Ext. Fel. Bovini 0.025 Gm., Bac. Acid. Lactic 0.05 Gm., Ext. Rham. Frangul. 0.025 Gm., Agar Agar 0.05 Gm. *Dose*: 1 or 2 tablets.

Nasciodine Brand Medicated Massage Cream (*A. Morgan Davis & Co. Ltd., London*). "A modern scientific preparation, analgesic, rubefacient. May be used effectively in the treatment of rheumatism, rheumatoid arthritis, lumbago, sciatica, neuritis, fibrositis, etc." *Formula*:—Iodine combined as Iodides 1.10, Methyl. Sal. 4.28, Menthol 1.07, Ol. Terebinthe Rect. 4.28, Ol. Camph. Rect. 4.28, Cera Alba 4.28, Emuls. Sap. Base.

[P1] **Nasmint Brand** (*Genatosan Ltd., Loughborough*). "Germ killing snuff. Cures and prevents colds." *Formula*:—Boric Acid 79%, Benzocaine 10.3%, Lactose 5%, Talcum Powder 4.7%, Menthol 1%.

Natex Slimming Food (*Modern Health Products Ltd., London*). "Natex Food No. 5. Reduce as you eat. A unique combination of natural vegetable products. It definitely does not contain any thyroid extract or drugs." *Formula*:—The sole ingredients during wartime are Rhubarb (root and stalk) 50%, Irish Moss 20%, Spinach 10%, Celery 10% and Dulse 10%, which are treated by Dr. J. Wigelsworth's dehydrating process and highly concentrated, so that their natural virtues are unimpaired.

Nature's Herbal Ointment (*O. Phelps Brown, Huddersfield*). "An external preparation for skin ailments. Very efficacious in cases of inflammation." *Formula*:—Bittersweet 9 lbs., Skunk Cabbage 14 lbs., Muscatels 15 lbs., Lard 56 lbs., Yellow Mollie 14 lbs., Resin 8 lbs., Lobelia Herb 14 lbs., Green Orzier Bark 6 lbs., Comfrey Leaves 10 lbs., Mutton Suet 10 lbs., Yellow Beeswax 8 lbs., Colouring q.s.

Nemakoi Brand Nasal Compound (*International Chemical Co. Ltd., London*). "For the prompt relief of head colds, catarrh, nasal congestion, hay fever and other nasal affections." *Formula*:—Ephedrine 0.75, Menthol 0.5, Camph. 0.5, Eucal. 0.5, Oleum Ricini 0.5, Liq. Par. q.s. 100.

Nemolin Pile Ointment (*Saltrates Ltd., London*). "Guaranteed treatment for piles, hæmorrhoids, pruritus and other rectal disorders. Antiseptic, soothing, non-astringent, allays itching." *Formula*:—Zinc Ox. 19%, Amylum 18%, Glycerinum 2%, Hamamelis 15%, Paraffinum 46%.

Nervone (*New Era Treatment Co. Ltd., London*). "Nervone is a vital cell-food which must be present in the blood to ensure complete health. In the treatment of brain exhaustion, sleeplessness, want of energy, lack of confidence and all nervous ailments, it is years ahead of ordinary methods." *Dose*: 2 tablets. The composition of this preparation was not disclosed.

N.E.T.C.O. 444 Pills (*New Era Treatment Co. Ltd., London*). "For stomach, liver and bowels. A herbal pill giving an easy natural motion without pain." *Formula*:—Podoph. Res. 6.25%, Sap. Dur. 7.71%, Aloin 6.25%,

Gentian 15.42%, Colocynth 10.28%, Zingib. 7.71%, Jalap Res. 2.57%, Capsic. 2.125%, Ipecac (sine Emet.) 3.125%. *Dose:* 2 to 6 pills.

[P1] **Nestosyl Brand Ointment** (*The Nestosyl Co. Ltd., Distributors: Bengue & Co. Ltd., Wembley*). "The affections for which Nestosyl ointment is most frequently prescribed are:—burns, scalds, eczema, varicose or cancerous ulcers, anal or vulvar pruritus, senile pruritus, infantile dermatitis, prurigo, diabetic pruritus, hemorrhoids, chilblains, chaps, sunburn, eschars, radio dermatitis, herpes zoster, erysipelas, boils, carbuncles, plegmons, impetigo, urticaria, herpes, blisters, intertrigo, insect bites, cracked nipples, vaginismus, vulvitis, Bartholinitis, papilloma, phimosis, painful dressings, post-operative wounds." *Formula:*—Approximate percentages: Ethyl-aminobenzoate 2.00, N-butyl-1-aminobenzoate 2.00, Resorcin 2.00, Zinc Oxide 10.00, 8-Oxyquinoline 0.006, Excipient q.s. for 100.

[P1] **Neuro Phosphates (Eskay Brand)** (*Distributors: Menley & James Ltd., London*). "A palatable and efficient tonic. To be taken as directed by the physician." *Formula:*—Each dose (two fluid drachms) contains in acid state: Sodium Glycero-phosphate 2 grains (1.667% w/v), Calcium Glycero-phosphate 2 grains (1.677% w/v), Strychnine Glycero-phosphate $\frac{1}{10}$ grain (0.013% w/v). *Dose:* Adults, 2 teaspoonfuls; Children, according to age.

New-Skin (New-Skin Co. Ltd., Hayward's Heath). "Antiseptic waterproof coating for cuts, scrapes and abrasions." *Formula:*—Nitrocellulose 6.9, Camphor 0.8, Ethyl Acetate 57.1, Butyl Alcohol 5.0, Oleum Ricini 3.0, Ethyl Alcohol 24.7, Amyl Acetate 2.0, Perfume 0.5.

New-Sphagnol 10% Ointment (*Peat Products (Sphagnol) Ltd., London*). "For Dispensing. For acne, chilblains, herpes, impetigo, eczema, pruritus, psoriasis, ringworm, sore feet, and skin irritations." *Formula:*—Pix Liquida 9% w/w, Xylenols 1% w/w.

Niblett's (Dr.) Vital Renewer (*C. P. Niblett, Richmond*). "Nerve Sedative." *Formula:*—Liq. Aurantii pro Tinct. 1-3 0.10, Tinct. Cinnamonii 0.55, Tinct. Calumbæ 0.415, Tinct. Lavandulæ Co. 0.075, Chloroform 0.15, Potassium Bromide 26.50, Ammonium Bromide 8.75, Potassium Iodide 4.40, Potassium Carbonate 0.075, Sacch. Ust. 1.38, Aqua ad 100.00. *Dose:* 1 teaspoonful.

Nigroids (Ferris & Co. Ltd., Bristol). "For throat and voice." Containing Ext. Glycyrrhiz. 68.6%, Menthol 2.06% (with flavouring).

Nipits (*The manufacturer's name is not given on the container*). "Voice, throat and chest pastilles. Prepared from 99.314 Pastille Base, .392 Menthol, .2938 Eucalyptus, .0002 Peppermint and Oleo-resin of Ginger."

Nixoderm (Knox Laboratories, London). "Treatment for skin troubles such as eczema, itching skin, cracking, peeling, burning skin, rash, ringworm, pimples, blackheads and foot itch." *Formula:*—Acid. Benzoic 12%, Acid. Salicyl. 3.5%, Zinc Oxide 10%, Sulphur. Precip. 4.57%, Talc. 4%, Menthol 1%, Petrol Molle Alb. 64.93%.

[P1-S7] **Nonn Brand Tablets** (*N. N. Co. Ltd., London*). "For men. Contain the extracts necessary to keep the internal glands of secretion normal, thus ensuring perfect health, virility and long life." *Formula:*—Suprarenalis 28.00, Orchis 15.00, Thyroidii 1.00, Prostatine 11.00, Pepsinæ 5.00, Sodii Bicarb. 23.00, Excipient ad 100.00. *Dose:* 1 tablet.

Normacol Brand Intestinal Evacuant (*Norgine Pharmaceutical Products (London) Ltd.*). "A purely vegetable product, not a laxative but a colon regulator." *Formula:*—Desiccated Bassorin 69%, Sugar 30.8%, Frangula Glucosides 0.12%, Peppermint 0.08%. *Dose:* 1 or 2 heaped teaspoonfuls.

Normo Gastrine Brand Tablets (*Burgoyne Burbridges & Co. Ltd., London*). "The new scientific treatment for excess acidity (indigestion). The unique feature of Normo Gastrine, which lifts it to a plane above all other antacids, is that it neutralises only the excess of acid." *Formula:*—Silicate of Aluminium 71.3 parts, Flavouring q.s., Excip. ad 100 parts. *Dose:* 1 or 2 tablets.

Norton's Brand Chamomile Pills (*John Bell, Hills and Lucas Ltd., London*). "A certain cure of indigestion, constipation, bilious liver and all stomach complaints." *Formula:*—Each pill contains Zingib. 0.12 gr., Myrrh. 0.16 gr., Rhei 0.33 gr., Sodii Bicarb. 0.1 gr., Aloe 0.37 gr., Ext. Anthem. Ang. 0.25 gr., Potass. Sulph. 0.9 gr., Cambog. 0.016 gr., Ol. Anthem. Ang. q.s. Excipient q.s. *Dose:* 4 or more pills.

Nostroline Brand Nasal Remedy (*Matthew's Laboratories Ltd., Bristol*). "For nasal catarrh, cold in the head, influenza, sore throat and hay fever." *Formula:*—Vegetable Stearine 5.970, Boric Acid 3.981, Cineol 0.244, Petro-latium 87.562, Menthol 0.313, Phenol 1.656, Geranium Oil 0.274 per cent.

Novaseptic Brand Germicide (*Pharmaceutical Mfg. Co. Ltd., Cheltenham*). "A powerful non-poisonous germicide and antiseptic for open wounds, burns, skin eruptions and affections of the mouth, throat and nose." *Formula*:—Capryl-hydrocupreinotoxin Hyd. 0.13% w/v, Acid Benz. 0.2% w/v, Acid Hydrochlor. Dil. B.P. 0.12% w/v, Perfume and Flavouring Agents 0.014% w/v in saline solution.

[P1] **Noxacorn** (*Thomas Marns Ltd., London*). "Antiseptic corn remover. For corns, callouses, warts." *Formula* w/v:—Benzocain 2.1%, Camphor 2.1%, Salicylic Acid 10.6%, Iodine 0.1%, Collodion to 100.

Nujol (*Stemco Ltd., London*). "Clear as crystal. Absolutely pure and harmless." *Formula*:—A blend of petroleum hydrocarbons. *Dose*: 1 tablespoonful. Children, $\frac{1}{2}$ to 3 teaspoonfuls.

Number 350 Brand Tablets (*The manufacturer's name is not given on the container*). "For rheumatism." *Formula*:—Acid. Acetylsalicyl. gr. 2.5, Amylum gr. 0.5, Tartrazine gr. 0.004. *Dose*: 2 tablets. In acute cases, twice this dose.

Numol (*Numol Ltd., Newcastle-on-Tyne*). "The lecithin food for body building. Tonic nerve, digestive, body building, pleasant to take." *War-time Formula*:—Extractum Malti 82.00%, Fats 4.00%, Sucrose 9.80%, Lecithinum 0.22%, Calc. Hypophosph. 0.19%. The Lecithinum may be ex-ovo or brain or ground nut. *Dose*: Infants, $\frac{1}{2}$ to $\frac{1}{2}$ teaspoonful; Children, $\frac{1}{2}$ to 1 teaspoonful; Adults, 1 to 2 teaspoonfuls.

Nurse Harvey's Mixture (*O. Scruton & Co., York*). "It instantly relieves wind spasms, griping pains and acidity; corrects possetting or sickness; prevents fevers, fits, convulsions and diarrhoea." *Formula*:—Oleum Anethi m. 3, Oleum Cari m. 3, Tinctura Zingiberis Mitis m. 150, Sodii Bicarbonas grs. 35, Syrupus m. 480, Aqua Puræ to 6 fluid ounces. *Dose*: Infants, $\frac{1}{2}$ to 2 teaspoonfuls. Children, 3 to 4 teaspoonfuls.

Odds On Liniment (*The "Odds On" Specifics Co. Ltd., Thames Ditton*). "For rheumatism, lumbago, neuritis, neuralgia, sciatica, frostbite, chilblains, colds, gouty eczema, sprains, cramp, etc." *Formula*:—Ol. Lini. Pur. 28.35 c.cs., Ol. Camphoræ alb. 0.0567 litres., Ol. Terebinthin Rectif. 56.70 c.cs., Ol. Amygd. Essential 0.2835 c.cs., Ol. Ti-tree 1.768,000.

[P1] **Okasa Brand Tablets** (*Lewis & Melchior Ltd., London*). "Gold for women." *Formula*:—Each tablet contains: Stilboestrol $\frac{1}{1000}$ gr., Orchitic Gland, dry $\frac{1}{2}$ gr., Cerebrin $\frac{1}{2}$ gr., Potassium Bromide B.P. $\frac{1}{2}$ gr., Vitamin A 300 units, Vitamin D (Calciferol) 200 units, Strychnine Hydrochloride B.P. $\frac{1}{1000}$ (0.001%) gr. *Dose*: 2 to 3 tablets.

[P1] **Okasa Brand Tablets** (*Lewis & Melchior Ltd., London*). "Silver for men. Made to Dr. Lahusen's formula." *Formula*:—Per tablet: Orchitic Gland, dry gr. $\frac{1}{2}$, Cerebrin gr. $\frac{1}{2}$, Potassium Bromide B.P. gr. $\frac{1}{2}$, Vitamin A 300 units, Vitamin D (Calciferol) 200 units, Strychnine Hydrochloride B.P. $\frac{1}{1000}$ (0.001%) gr. *Dose*: 2 to 3 tablets.

[P1] **Omega Oil** (*Omega Chemical Co., London*). "For the treatment of rheumatism, lumbago, sciatica, pains in the joints, tender feet, weak backs, swellings, bruises, sprains, stiffness, inflammations, sore throat, cold in the chest, bronchitis, quins, asthma, neuralgia, etc." *Formula*:—Paraff. Liq. 71.4% v/v, Methyl Salicyl. 20% v/v, Chlorof. 8.6% v/v, Solanaceous alkaloids, calculated as hyoscyamine, 0.002% w/v.

[P1] **One Day Cold Cure** (*The Mackenzie Medicine Co. Ltd., London*). "A speedy cure in all cases of cold, influenza, headache and all neuralgic affections." *Formula*:—Camphor $\frac{1}{2}$ gr., Capsici $\frac{1}{2}$ gr., Caffeine $\frac{1}{2}$ gr., Acetanilide 1 gr., Podophyllin Resin $\frac{1}{2}$ gr., Aloin $\frac{1}{2}$ gr.

Opas Brand Maclean Powder (*Wigglesworth Ltd., Westhoughton*). "Prescribed to relieve and cure indigestion, gastritis, ulcerated stomach, flatulence, acidity, etc." *Formula*:—Sod. Bicarb. 18.19, Mag. Carb. Pond. 36.36, Calc. Carb. 36.36, Bism. Carb. 9.09. *Dose*: 1 teaspoonful.

Opas Tablets (*Wigglesworth Ltd., Westhoughton*). "Stomach digestive tablets for acidity, gastritis, wind, dyspepsia." *Formula*:—Each tablet contains approximately: Maclean Powder:—Sod. Bicarb. 5.26, Mag. Carb. Pond. 10.53, Calc. Carb. 10.53, Bism. Carb. 2.63; together with Sucros 57.89, Lactos 10.53, Lubricant 2.63, Flavour q.s. *Dose*: 2 or 3 tablets.

Optrex Brand Eye Lotion (*Optrex Ltd., Greenford*). "For daily ocular hygiene; eyestrain; tired eyes; eyes affected by light; watering, redness, irritation, black spot, etc. Inflammation and congestion of the eye, and eyelids; conjunctivitis, styes, blepharitis, hay fever, colds in the eye." *Formula*:—Liq. Ext. Potentill Erect. 0.5 gm. Liq. Ext. Polyg. Bistort 0.5 gm., Liq. Ext. Erig.

Canad. 0.5 gm., Liq. Ext. Euphras. Off. 0.5 gm., Liq. Ext. Hamam. Virgin 0.5 gm., Acid Boric 2 gm., Sod. Borate 0.5 gm., Sod. Salicyl. 0.08 gm., Zinc Sulph. 0.1 gm., Liquor Hamam. Virgin 25 gm., Aq. Destill. ad 100 gm.

[P1] **Orlex Compound** (*Orlex Mfg. Co., Welwyn Garden City*). "For making a grey hair restorer. An excellent hair tonic, removes dandruff, and stops the hair from falling out." *Formula*:—33 $\frac{1}{3}$ % weight in weight of this compound is Lead Acetate.

Osborne's Mixture (*James Osborne, Ashbourne*). "Nerve sedative. Strengthens the system, purifies the blood and improves the general health." *Formula*:—Pot. Brom. 23%, Sod. Brom. 7.33%, Inf. Calumb. 3.33%, Inf. Gent. 3.33%, colour and flavour q.s. *Dose*: 1 teaspoonful. Children, $\frac{1}{4}$ to $\frac{1}{2}$ teaspoonful.

Owbridge's Lung Tonic (*W. T. Owbridge, Hull*). "For coughs, colds, asthma and other similar disorders." *Formula*:—Capsicum .002, Ol. Anisi .044, Ol. Caryoph. .049, Ether .333, Senega .066, Sp. Rect. 1.003, Mel. 32.917, Chlorof. .866, Acetum 10.125, Sucrose 36.326, Aq. ad 100. *Dose*: $\frac{1}{2}$ to 1 teaspoonful (or less).

Oxien Brand Nerve Tablets (*The Giant Oxie Co. Ltd., London*). "Give strength, health and vigour to young and old. Nothing equals these Oxien Nerve Tablets in nerve troubles, digestive complaints and minor functional heart troubles." *Formula*:—Lecithin 0.63, Bism. Carb. 1.26, Mag. Carb. Pond 2.52, Ferr. Hypophosph. 1.26, Tragacanth 0.10, Amylum 5.07, Acacia 13.52, Talc. Pur. 3.33, Sucros. 67.16, Ol. Sassaf. 0.44, Ol. Betul 0.28, Gentian 0.12, Eupatorium 0.12, Elecampane 0.12, Scutellaria 0.12, Chirata 0.98, Colour 2.97. *Dose*: 1 tablet.

Oxien Brand Pills (*The Giant Oxie Co. Ltd., London*). "For headaches, constipation and indigestion. Create an active liver. If used in connection with Oxien Nerve Tablets, they will aid in the cure of nervous troubles and ailments due to weakened heart, nerves and blood." *Formula*:—Aloin gr. $\frac{1}{4}$, Podoph. Res. gr. $\frac{3}{32}$, Ext. Gent. gr. $\frac{1}{4}$, Camphor gr. $\frac{1}{32}$, Ext. Jalap. gr. $\frac{1}{4}$, Capsicin (Water Soluble) gr. $\frac{1}{16}$. *Dose*: 2 pills.

Oystrax Brand Tonic (*Oystrax Ltd., Morecambe*). "Recommended for rundown condition, general weakness, nervousness, underweight, anæmia, low vitality, early old age." *Formula*:—Vitamin B₁ (Thiamine Hydrochloride) 25 International Units, Exsiccated Ferrous Sulphate 1.5 grains, Calcium Phosphate 3 grains. *Dose*: 2 tablets.

Pacific Pain Balm (*W. B. Cartwright Ltd., Rawdon*). "The new treatment for neuralgia, headache, lumbago, stiff joints, gout, etc." *Formula*:—Paraff. Moll. Alb. 12.1, Cera Alb. 3.75, Methyl Salicyl. 5.9, Menthol 1.1, Borax 0.35, Aqua Dest. 7.6.

Page Woodcock's Wind Pills (*Page Woodcock Ltd., London*). "For indigestion, liver complaints, wind on the stomach, biliousness." *Formula*:—Each pill contains: Aloes $\frac{1}{32}$ rd gr., Ext. Gent. $\frac{1}{32}$ rd gr., P. Zingib. $\frac{1}{16}$ th gr., P. Myrrh $\frac{1}{16}$ th gr., P. Gentian $\frac{1}{32}$ rd gr., P. Calumb. $\frac{1}{16}$ th gr., Ol. Anthem q.s., Ol. Menth. Pip. q.s., Ol. Cassia q.s. *Dose*: 2 pills.

Panacel Brand Antiseptic Ointment (*Belle Isle Laboratories Ltd., London*). "For wounds, cuts, abrasions, insect bites and stings, chilblains, chapped skin, cold sores and most skin troubles." *Formula*:—Sodii Hypochloris .1%, Sodii Chloridum 1.65%, Sodii Chloras .01%, Potassii Permanganas a trace, Dichloramina 1%, Magnesii Hydroxidum 2%, Calcii Carbonas 3%, Paraffinum 84%, Aqua 8.24%.

[P1] **Parmint Double Strength Concentrated Essences** (*International Laboratories Ltd., London*). "A modern scientific treatment for coughs, colds, bronchitis, whooping cough, catarrh, sore throat and all chest and lung troubles." *Formula*:—Tr. Capsici Min. Aquosa 6.7%, Tr. Camph. Co. (Sine Opio) 33.3%, Ext. Urginea Scillæ Liq. 3.3%, Invert Syrup 23.37%, Liq. Tolu (Myroxylon) 1.7 10.0%, Tr. Cephælis Ipecac. 16.7%, Chloroform 1.7%, Ol. Menth. Pip. 1.8%, Ol. Pimpinell. Anis. 0.3%, Ol. Pini Sibiric 0.03%, Sod. Benzoate 2%, Liq. Dactylopii Cocci 0.8%. *Dose*: 1 teaspoonful.

[P1] **Parmint Syrup** (*International Laboratories Ltd., London*). "Ready for use. An invaluable specific for coughs, colds, catarrh, head noises, bronchitis, whooping cough, influenza, laryngitis, catarrhal deafness." *Formula*:—Tr. Capsici Min. Aquosa 0.67%, Tr. Camph. Co. (Sine Opio) 3.33%, Ext. Urginea Scillæ Liq. 0.33%, Glycerol 2.58%, Liq. Tolu (Myroxylon) 1.7, 1.00%, Tr. Cephælis Ipecac. 1.67%, Chloroform 0.17%, Ol. Menth. Pip. 0.18%, Ol. Pini Sibiric 0.003%, Ol. Pimpinell. Anis. 0.03%, Liq. Dactylopii Cocci 0.08%

Sodium Benzene Carboxylate 0.2%, Syrupus Simplex 89.75%. *Dose*: 1 tablespoonful. Children, $\frac{1}{2}$ to 1 teaspoonful.

Passorine Brand Sedative (*Bengue & Co. Ltd., Alperston*). "Employed in disorders of the nervous system, neuropathic conditions, vertigo, anxiety, nervous insomnia, cardiac troubles, etc." *Formula*:—Fluid Extract of Passiflora Incarnata, approximate percentage 10, Extract of Salix Alba 5, Alcoholic Tinct. of Crataegus Oxyacantha 26, Syrup and Glycerine *q.s.* *Dose*: 1 to 4 teaspoonfuls. [P1] **Paternoster's Gout and Rheumatic Pills** (*Poingdestre & Truman, London*). "For gout, rheumatism and rheumatic gout; for influenza and severe colds; for liver affections, as a general aperient pill." *Formula*:—Acacia 9.75%, Aloe 7.00%, Antim. Sulphur 1.13%, Camphora 10.33%, Guaiac. Res. 10.33%, Jalap. Pulverat. 7.00%, Rheum 28.14%, Scilla 2.60%, Ext. Colch. Sem. 8.00%, Ext. Lact. 14.00%, Ol. Junip. 1.72%. *Dose*: 1 or 2 pills.

Patterson's Pills (*Hygienic Stores Ltd., London*). *Formula*:—Ferrous Sulph. Exsic. 10%, Aloes 20%, Pulv. Cinnamon Zeylanicum 12%, Pulv. Sem. Eleuteraria Cardam. 12%, Pulv. Rhizome Zingiberis 12%, Liq. Syr. Glucose 34%, Ol. Mentha Pulegium *q.s.* *Dose*: 2 pills.

Pellanthum (*Handford & Dawson Ltd., Harrogate*). "For treatment of eczema and all skin affections." *Formula*:—The high therapeutic value of this preparation is due to its containing: Zinc Carb., Zinc Oxid. aa 11.9%, Zinc Stear. 5.9% approx., Thymol 0.23%, combined in readily absorbed base.

Penetrol Brand Inhalant (*W. B. Cartwright Ltd., Rawdon*). "Preventive against catarrh, influenza, head colds, hay fever, headache, etc." *Formula*:—Menth. 7.41%, Ol. Lavand. 17.60%, Ol. Cajaput. 5.86%, Ol. Menth. Pip. 0.22%, Otto Lavand. 4.32%, Ol. Eucalypt. 6.41%.

Penetrol Brand Tablets (*W. B. Cartwright Ltd., Rawdon*). "Promptly relieves asthma, bronchitis, hay fever, catarrh, difficult breathing, coughing spasms, wheeziness." *Formula*:—Ephed. Hydrochlor. B.P. 0.02 gm. Calc. Gluconate 0.0075 gm. Theobromine 0.04125 gm. Antipyrine 0.08 gm., Excipient and Colour *q.s.* *Dose*: 1 or 2 tablets. Children, $\frac{1}{2}$ to $\frac{1}{4}$ tablet.

Peps Brand Pastilles (*Peps Pastille Co., London*). Ol. Res. Cubeb. 0.015 m., Ol. Menth. Pip. 0.075 m., Ol. Anisi. 0.075 m., Ol. Eucalypt. 0.075 m., Bals. Tolut. 0.150 gr., Ext. Pini Canad. Liq. 0.015 m., Ext. Capsici Liq. 0.015 m., Ext. Tussilag. Liq. 0.575 m., Ext. Glycyrr. 2.250 m.

Petrolagar Brand Paraffin Emulsion (*John Wyeth & Brother Ltd., London*). "No. 1 Plain. For the treatment of constipation. It is entirely mechanical in action and successfully replaces purgative medication." *Formula*:—Contains 25% (volume) of medicinal Liquid Paraffin. *Dose*: 1 dessertspoonful. Children, 1 teaspoonful.

Petrolax Compound (*C. R. Harker, Stagg & Morgan, London*). "An eminently efficient intestinal lubricant together with the laxative effect of Phenolphthalein." *Formula*:—The active ingredients of this preparation are Paraffin Liq. B.P. 25% v/v, Phenolphthalein 1.375%, Agar 0.333%.

Pharmalene (*The Proctor Medicinal Co., Stockport*). "Ointment and embrocation for rough skin, blemishes, freckles, chafes, or tender skin, abrasions, etc." The composition of this preparation was not disclosed.

[P1] **Phenalgine** (*Etna Chemical Co., London*). "Invaluable for the relief of pain, headache, neuralgia, sleeplessness, painful or scanty menstruation, etc." *Formula*:—Acetanilide 49% w/w, Sodii Bicarb. 25% w/w, Ammonii Bicarb. 26% w/w. *Dose*: 1 to 4 tablets.

[P2] **Phenoda Solution** (*James Woodley, Manchester*). "As a mouth-wash, gargle or antiseptic dressing. For wounds, burns, scalds, chilblains, stings, bruises, swellings, etc." *Formula*:—Phenol 10% w/v, Glucosum 4.16%, Sodii Hydrox. 1.07% w/v, Aqua ad 100%.

[P1] **Phenolaine Ointment** (*The Phenolaine Co., Goudhurst*). "For cuts, abrasions, burns, piles, pruritus, irritations, chilblains, eczema, pimples and boils." *Formula*:—Phenolaine minims 24, Petroleum Jelly ounces 1.

Phensic Brand (*Veno Drug Co. Ltd., Manchester*). "For headaches, nerve pains, neuralgia, neuritis, rheumatism, colds and influenza." *Formula*:—Acidum Acetylsalicylicum 48.18%, Acetphenetidinum 27.40%, Caffeina 6.57%, Salicinum 3.58%, Creta Gallica 5.00%, Amylum Zea Mays 5.84%, P. Acaciae Gummi 3.20%, Ol. Limonis 0.23%. *Dose*: 2 tablets.

Phillips Pure Live Tonic Yeast Tablets (*Phillips Yeast Products Ltd., London*). "The most active and pure preparation very rich in vitamin B. A safe and sure remedy for indigestion and nerve troubles." *Formula*:—4 grain Yeast tablets. *Dose*: 2 tablets; Children, 1 tablet.

Phillips Pure Live Yeast Granules (*Phillips Yeast Products Ltd., London*). "Excellent for indigestion, stomach troubles, rheumatism, nerve troubles, lack of energy, anemia, impure blood, skin eruptions, constipation."

Phosferine (*Phosferine (Ashton & Parsons) Ltd., Watford*). "For weakness, nervousness, lassitude, debility, loss of appetite and rheumatism. For neuralgia, toothache and other severe nerve pains." *Formula*:—Acid Phosph. Conc. 8.28%, Acid Glycerophosph. 0.063%, Sodii Phosphas 1.0%, Tinct. Quassia 6.0%, Saccharum Ustum 0.25%, Aqua 84.407%. *Dose*: 5 to 10 drops.

Phosferine Health Salts (*Phosferine Products Ltd., London*). "Tonic effervescent saline. It will be found invaluable for disorders of the liver, sick headache, biliousness, rheumatism, lumbago and uric acid troubles, constipation, irregularity, and digestive ailments." *Formula*:—Sodium Bicarbonate 49.402%, Acid Citric 47.301%, Acid Tartaric 2.210%, Sodium Phosphate 1.087%. *Dose*: 1 teaspoonful. Children, $\frac{1}{2}$ to $\frac{1}{2}$ teaspoonful.

Phosferine Tablets (*Phosferine (Ashton & Parsons) Ltd., Watford*). "For weakness, nervousness, lassitude, debility, loss of appetite and rheumatism. For neuralgia, toothache and other severe nerve pains." *Formula*:—Acid Phosph. Conc. 11.25%, Acid Glycerophosph. 0.015%, Ext. Quassia 0.75%, Ol. Limonis 0.625%, Glycerin Substitute 13.5%, Sodii Phosphas 0.75%, Gelatin 73.11%. *Dose*: 1 or 2 tablets.

Phyllosan (*Natural Chemicals Ltd., London*). "To revitalise the blood and fortify the heart, relieve arterial tension, improve the circulation, strengthen the nerves, stimulate metabolism and increase the vital forces." *Formula*:—Chlorophyll B.P.C. 0.01 g. (approx.), Ferri Phosphas B.P.C. 0.01 g., Sacrosum B.P. 0.10 g., Calcii Phosphas Dibasic 0.05 g. *Dose*: 2 or 3 tablets.

Physikurate (*R. C. Johnson Ltd., Grimsby*). "For rheumatism, lumbago, neuritis, sciatica, gout." *Formula*:—Sucros 11%, Pot. Cit. 15%, Sod. Bicarb. 43%, Acid. Tart. 21%, Acid. Cit. 10%. *Dose*: 1 teaspoonful.

Pierre's (Father) Monastery Herbs (*Monseaton Herbalists Ltd., Salford*). "Nature's cure for constipation, rheumatism, blood pressure, indigestion, etc. A wonderful combination of herbs, barks, seeds and flowers." *Formula*:—Each ounce contains Sacred Bark 25.73 gr., Frangula 6.43 gr., Foeniculum 51.45 gr., Senna Folium: Cassia Angustifolia 157.63 gr., Cassia Acutifolia 64.36 gr., Urtica Dioica 12.86 gr., Rubus Villosus 8.09 gr., Mate Folia 25.73 gr., Taraxacum Officinale 12.86 gr., Ispaghula 25.73 gr.

Pineate Brand Honey Cough Syrup (*Bismar Ltd., London*). "A concentrated bronchial remedy that soothes and relieves." *Formula*:—Oil of Peppermint 0.26, Oil of Pine 0.24, Chloroform 0.4, Menthol 0.1, Liq. Scillae 3.25, Liq. Tolu 3.6, Ext. Ipecac. Liq. 0.37, Honey Syrup to 100. *Dose*: 1 teaspoonful. Children, $\frac{1}{2}$ teaspoonful.

Pinelyptus Brand Pastilles (*Proctor's Pinelyptus Depot, Newcastle-on-Tyne*). "For relief of husky voice, minor throat irritations and coughs due to colds." *Formula*:—Menthol 0.548, Ol. Eucalypti 0.842, Ol. Pini Pumilionis 0.240, Glusidi 0.003, Gelatin 2.0, Glucose 24.0, Sucrose 30.0, Acacia 42.367.

Pineoleum Brand Ephedrine Jelly (*Chesebrough Mfg. Co., London*). "Specially recommended for head colds and nasal congestion." *Formula*:—Ephedrine Alkaloid .50, Menthol .50, Camphor .50, Ol. Eucalyptus Glob. .56, Ol. Pini Pumilio 1.00, Ol. Cassia .07, Petrolatum 96.87.

Pineoleum Brand Inhalant (*Chesebrough Mfg. Co., London*). "For the relief of catarrhal conditions of the mucous membranes of the nose and throat." *Formula*:—Menthol .50 Gm., Camphor .50 Gm., Ol. Eucalyptus Glob. .56 Gm., Ol. Pini Pumilio 1.00 Gm., Ol. Cassia .07 Gm., Chlorophyll .12 Gm., Liq. Petrolatum 97.25 Gm.

Pineoleum Brand Inhalant with Ephedrine 0.5% (*Chesebrough Mfg. Co., London*). "For the relief of catarrhal conditions of the mucous membranes of the nose and throat." *Formula*:—Menthol .50 Gm., Camphor .50 Gm., Ol. Eucalyptus Glob. .56 Gm., Ol. Pini Pumilio 1.00 Gm., Ol. Cassia .07 Gm., Chlorophyll .12 Gm., Liq. Petrolatum 97.25 Gm. With Ephedrine 0.5%.

Pinkettes Brand Tiny Laxative Pills (*The Dr. Williams Medicine Co., London*). "For constipation, biliousness, dizziness, sick headache, torpid liver, pimples and eruptions, offensive breath, coated tongue." *Formula*:—Aloin 50%, Resin. Podophylli 6.66%, P. Ipecac. 10%, Oleoresin Zingiber 6.66%, Excip. 26.68%. *Dose*: Laxative, 1 pill; Cathartic, 2 pills; Purgative, 3 pills.

[P1] **Platts (Dr.) Rinex Prescription** (*Distributors: Brooks & Warburton Ltd., London*). "For the paroxysms of asthma and nasal congestion and irritation of hay fever, nasal catarrh, head colds." *Formula*:—Powdered

Capsicum $\frac{3}{4}$ gr., Acetanilid 1 gr., Caffeine Alkaloid $\frac{1}{10}$ gr., Extract Euphorbia $\frac{1}{10}$ gr., Extract Cascara Sagrada $\frac{1}{4}$ gr., Quinine Salicylate $\frac{1}{4}$ gr., Powdered Camphor $\frac{1}{8}$ gr., Extract Stramonii Fol. $\frac{1}{16}$ gr., Acetyl Salicylic Acid 2 $\frac{1}{2}$ gr., approx. in each capsule, Calcium Carbonate 5 gr. approx. in each tablet. *Dose:* 1 or 2 capsules and 1 tablet.

[P1] **Pomade Max** (May Roberts & Co. Ltd., London). "For loss of hair and baldness. Used in Alopecia prematura, Alopecia areata, Alopecia seborrhoica and other forms of loss of hair." *Formula:*—Contains Liq. Epispast P.B. 0.25 in 100 and 1 in 3 of Ung. Hyd. Nit. P.B.

Pond's Extract Co. Ointment (Pond's Extract Co. Ltd., London). "Invaluable for piles, also burns, sunburns, stings, scalds, ulcers, chapped hands, eruptions, etc." *Formula:*—Cera Flav. 24.00%, Petrolatum Liq. 41.00%, Paraffin Molle 19.00%, Menthol 4.50%, Camphor 0.40%, Pulv. Galla 11.00%, Ess. Bay 0.10%.

Pond's Extract of Hamamelis (Pond's Extract Co., Ltd., London). "For piles, toothache, headache, earache, sore throat, sore eyes, nose bleed, stings of insects, neuralgia, hæmorrhages, inflammations." *Formula:*—85% Distilled Extract of Hamamelis, 15% Rectified Spirits of Wine.

Poor Man's Friend (Beach & Barnicott, Bridport). "For ulcerated sore legs (even if of twenty years' standing), and wounds and skin eruptions, burns, scalds, chilblains, etc." *Formula:*—Hydrarg. Sabetlor 3.5%, Plumbi Acet. 1.255%, Red Powder 35%, Perfume .175%, Bees Wax 7%, Lard 7.5%.

Potter's Asthma Cigarettes (Potter & Clarke Ltd., London). "For the relief of asthma, bronchitis, catarrh, hay-fever and spasmodic diseases of the respiratory apparatus." *Formula:*—Stramonium 50%, Lobelia 18%, Tussilaginis Folium 20%, Anisum 10%, Potassii Nitras 2%.

Potter's Asthma Cure (Potter & Clarke Ltd., London). "For the relief of asthma, hay fever, bronchitis and diseases of the respiratory apparatus." *Formula:*—Stramonium 30%, Lobelia 15%, Tussilaginis Folium 30%, Anisum 7%, Potassii Nitras 18%.

Potter's Catarrh Pastilles (Potter & Clarke Ltd., London). "Cure coughs, colds, hay fever, bronchitis." *Formula:*—Oleum Pini Sylvestris 8, Oleum Pini Pumilionis 8, Oleum Eucalypti 4, Creosote 4, Ext. Althææ 10, Menthol 16, Thymol 4, Sucrose 800, Acacia 1,000 Aqua q.s.

Potter's Herbal Smoking Mixture (Potter & Clarke Ltd., London). "It will be found highly valuable in cases of bronchial affection, sore throat, catarrh, etc." *Formula:*—Coltsfoot Leaf 49%, Rose Petals 10%, Clover Flowers 30%, Lavender Flowers 10%, Perfume 1%.

Potter's Stramonium Cigarettes (Potter & Clarke Ltd., London). "For the immediate relief of asthma, bronchitis, hay fever, and spasmodic diseases of the respiratory organs." *Formula:*—Contains Stramonium 90%.

Presto Powders (W. Davies & Co. Ltd., London). "The new scientific remedy for the prompt relief and cure of headache, toothache, neuralgia, quinsy, swollen tonsils, influenza, gout and rheumatic affections. Are of purely vegetable origin." *Formula:*—Acid. Acetyl Salicyl. B.P. 64.28, Saccharum Purificatum 35.71, Ol. Limonis and colouring q.s.

[P1] **Proctoids** (John Wyeth & Bro., London). "Hæmorrhoidal Suppositories. Relieve pain and discomfort, reduce congestion and check bleeding." *Formula:*—Zinc. Ox. 10%, Ac. Boric. 10%, Bism. Oxyiod. 1.67%, Bism. Subcarb. 8.33%, Ext. Belladonnæ 0.5%, Ephedrin. Sulph. 0.1%, Bals. Peru 1%, Cera Flav. 5%, Ol. Theobrom. q.s. ad 100.

Propax Brand Tablets (Distributors: Splendor Ltd., London). "Relieve pain quickly." *Formula:*—Acid Acetylsalicyl gr. 3.5, Acetphenetidin gr. 2.0, Phenolphthal. gr. 0.166. *Dose:* 2 tablets.

Prunol Brand Pastilles (Prunol Proprietary Ltd., London). "For constipation." *Formula:*—Prunes 21.55%, Extr. Rhamnus pursh. 2.70%, Emodin comp. .50%, Anthragluco-sennin comp. 2.95%, Ol. Gingerine .01%, Excipient 72.29%. *Dose:* Adults, 1 to 2 pastilles. Children, $\frac{1}{2}$ pastille.

[P1] **Pulmo Baily (Concentrated)** (Bengue & Co. Ltd., Wembley). "Tonic reconstituent of the organism. Antisepsis of the respiratory tract. Cough sedative. Diminution of expectoration. Broncho-pulmonary affections. Coughs, colds, influenza, chronic bronchitis, catarrh, asthma, pleurisy, laryngitis, pharyngitis." *Formula:*—Ext. Pulmonaria 0.140 grm. approx., Ext. Carragahan 0.140 grm. approx., Guaiacol Pur. 2.253 grm. approx., Calcium Phosphoguaiacolate 0.140 grm. approx., Codeine Phospho-guaiacolate 0.281 grm. approx., Sodium Phosphoguaiacolate 0.140 grm. approx., Di-phosphoric acid 2.253 grm. approx., Glycerinated Excipient ad 100. *Dose:* 1 teaspoonful.

Purgen (*H. & T. Kirby & Co., London*). "The ideal purgative." Tablets containing phenolphthalein and supplied in three strengths: "Infant," $\frac{1}{4}$ gr; "Adult," $1\frac{1}{2}$ gr.; "Strong," $7\frac{1}{2}$ gr.

[P1] **Purgoids** (*Evans, Lescher & Webb, London*). "A safe laxative for chronic constipation." Formula:—Phenolphthalein 44.4%, Aloin 22.2%, Ipecacuanha Pulv. 5.5%, Ext. Bellad. Liq. P.B. 13.5% *wt/wt*. Dose: 1 or 2 tablets.

Puri-Lax (*S. Laycock, Eastbourne*). "Internal cleanser and blood purifier. Nature's herbal harmless remedy." Formula:—Senna Leaf, Fennel, Frangula, Mate, Elder Leaves, Balmony. Equal parts. Dose: $\frac{1}{2}$ teaspoonful. Children, $\frac{1}{4}$ teaspoonful.

Pylitna Aperient Tablets (*The Proprietors of Pylitna, London*). "A genuine and specific cure for hæmorrhoids or piles." Formula:—Ext. Casc. Sagr. Sicc. 2 gr., Aloe $\frac{1}{2}$ gr., Rheum $\frac{1}{2}$ gr., Ol. Res. Zingib. $\frac{1}{2}$ gr., Glycyrrh. $\frac{1}{2}$ gr. Dose: 1 or 2 tablets.

Pylitna Brand Pile Powders (*Manufacturers of Pylitna Preparations, London*). "Will speedily cure or prevent an attack. Will also prevent fistula." Formula:—Pot. Nitras 1.739%, Cubeba 6.956%, Glycyrrh 24.349%, Sulphur Sublim 13.913%, Mag. Carb. Pond. 13.913%, Pot. Tart. Acid 13.913%, Cascariil 11.304%, Acac. 13.913%.

[P1] **Pyorrhosol Brand** (*Pyorrhosol Ltd., London*). "Cures pyorrhœa. Also cures nasal catarrh, sore gums and prevents flu." Formula:—Veg. Inf. 10.00, Sod. Sulph. 2.50, Sod. Benz. 2.10, Acetanilid 1.15, Sod. Phen. 1.25, Aromat. Inf. 1.50, Ext. Coal Tar 7.50, Glycerol 4.00, Aqua Marina 70.00.

Pyridium Tablets (*Menley & James Ltd., London*). "It is advisable for the patient's sake, that Pyridium in any form, should be employed strictly in accordance with the physician's instructions." Formula:—12 tablets each containing 0.1 g. of Pyridium (Phenyl-azo-alpha-alphadiamino-pyridine hydrochloride).

Quinphos Brand Tonic (*Thompson & Capper Wholesale Ltd., Liverpool*). "The great tonic. For weakness, debility, loss of appetite, nerves, depression, sleeplessness, lack of energy, neuralgia, colds and influenza." Formula:—Quinquin Sulphas 4.68, Tc. Ferr. Perch. .078, Ac. Phosph. Conc. 15.37, Methyl Aldehyde .078, Aq. ad 100. Dose: 6 to 10 drops.

Radian α Brand (*W. Stevens & Co.'s Radiol Co., London*). "Spirit Dressing. Eases the pain and reduces the swelling in cases of arthritis of the joints with enlargement; bursitis, as in bunions and painful enlarged big toe swellings; also callosities and corns between the toes." Formula:—Benzol Mono Methyl Ether .0984, Methyl Isopropyl Cyclohexanol .0492, Trimethyl Dicyclo Heptanon .0164, Phenol Methyl Salicylate .0974, Ol. Citronella 0.0010, Aqua .0639, Glycerine .0819, Spirit (M.A.) .5918, Perfume *q.s.*

Radian β Brand (*W. Stevens & Co.'s Radiol Co., London*). "Aspirin Spirit Liniment. Relieves the pain of neuritis and rheumatism, also reduces inflammatory swellings." Formula:—Every 100 parts of Radian Liniment contains Methyl Isopropyl Cyclohexanol ($C_{10}H_{18}O$) Synthetic 03.5, Trimethyl Dicyclo Heptanon ($C_{10}H_{18}O$) Synthetic 00.8, Acidum Acetyl-Salicylicum 02.0, Methyl Salicylate 01.0, Ol. Camphore Essentielle 00.5, Ol. Citronella 00.3, Glycerine 04.0, Liq. Ammon. Ft. 00.2, Aqua 24.8, Alcohol 62.9.

"Radium" Buxton Crystal Salts (*Radium Ltd., Broadheath*). "The natural cure for rheumatism. Highly recommended by the Medical Faculty for general family use and in all cases of rheumatism, gout, lumbago, sciatica, dyspepsia, constipation, indigestion and all other disorders arising from the liver and kidneys." Formula:—Sod. Sulph. Crys. B.P. Pur. Dose: $\frac{1}{2}$ to $\frac{1}{4}$ teaspoonful.

Radox Bath Salts (*E. Griffiths Hughes Ltd., Manchester*). "Radiates oxygen. A balanced preparation which impregnates the water of the bath or hand basin with valuable salts, and supercharges it with oxygen, forming an artificial mineral water combining the properties of the world-famous medicinal spa waters of Carlsbad, Vichy, Marienbad, and similar baths. Immersion for 20 minutes eases rheumatic pains and is as beneficial as Spa treatment for lumbago, sciatica, gout, etc." Analysis:—Sodium Oxide (Na_2O) 38.8996, Boron Trioxide (B_2O_3) 4.3403, Carbon Dioxide (CO_2) 35.3310, Available Oxygen (O_2) 0.5192, Chloride (Cl) 0.2218, Water of Constitution (H_2O) 20.6881, this package containing per centum Sodii Pyroboras 10.3848, Sodii Bicarbonas 29.0442, Sodii Chloridum 0.3655, Sodii Percarbonas 5.1924, Acid. Boric. 0.9735, Sodii Carbonas, partially dehydrated quantum sufficit ad 100 approx.

Ralgex Brand Analgesic (*Pharmax Ltd., London*). "A solid radioactive analgesic embrocation for painful conditions of rheumatism, sciatica, lumbago, gout, neuralgia. Also catarrh, nephritic pains, sprains and all similar conditions." *Formula*:—Bromide of Mesothorium 0.096 micrograms, Glycol Salicylate 4 grms., Ethyl Salicylate 4 grms., Methyl Salicylate 1 grm., Capsicin 0 grm. 50, Menthol 6 grms., Excipient q.s. ad 100 g.

Raminal Brand Tablets (*H. R. Napp Ltd., London*). *Formula*:—Each "Raminal" tablet contains approx.: Theobromine-Calcium-Salicylate gr. 1½, Chlorophyll gr. ¼, Iron Phosphate gr. ¼. *Dose*: 1 or 2 tablets.

Rand's Aniska Brand Pills (*Aniska Preparations (Rand's) Ltd., Kingsdown*). "It quickly checks over production of uric acid and toxins in the digestive organs and by the action of its powerful medicaments, rapidly dispels them from the system." *Formula*:—Pulv. Aloes Curacao 40.0%, Pulv. Glycyrrh. Co. 18.2%, Pulv. Myrrhæ 9.1%, Bism. Oxycarb. 21.8%, Ext. Hamamelidis Liq. 2.0%, Excipient q.s. *Dose*: 1 pill.

Rankin's Ointment (*Rankin & Co., Kilmarnock*). "For control and destruction of nits and vermin." *Formula*:—Ext. Lonchocarpus Utilis 5%, Paraffin Ointment Base 95%.

Raspails' (Dr.) Female Pills (*The manufacturer's name is not given on the container*). "An effective and speedy remedy for female irregularities and complaints." *Formula*:—Each pill contains Sulphate of Iron 2 gr., Bitterapple ½ gr., and Pennyroyal Oil Min. ¼. *Dose*:—Not more than 3 pills during 12 hours.

Reade's Express Powders (*Reade Bros. & Co. Ltd., Wolverhampton*). "Give relief beyond belief. For severe headache, influenza, feverishness, chills, colds, neuralgia, toothache, hay fever, sciatica, rheumatism, etc." *Formula*:—Each powder contains: Acetphenetidin gr. 2.5, Caffein Alk. gr. 0.25, Acid. Acetylsalicyl gr. 4.75, Lactos, etc., ad gr. 16.

Red Band Brand (Bismuthated) Magnesia (*Red Band Chemical Co., Edinburgh*). "The simplest, safest and most efficacious remedy for indigestion, acidity, heartburn, gastric and duodenal ulcers." *Formula*:—Bismuth. Carb. 4.46%, Sodii Para Sulphonas 2.223%, Sodii Bicarb. 13.331%, Magnes. Carb. Lev. 80%. *Dose*: 1 teaspoonful in water or milk.

Red Band Cream of Magnesia Tablets (*Red Band Chemical Co., Edinburgh*). "Vitalised cream of magnesia with glucose. The wonderful family remedy for indigestion, flatulence, heartburn, acidity, constipation, impure blood, muddy complexion, lack of vitality." *War Restrictions Formula*:—Each tablet contains: Magnes. Hydrox. 50%, Glucose 25%, Magnes. Carb. Pond. 12½%, Paraffin Liq. Alb. 2%, Ol. Menth. Pip. 0.08%, Talc 10.42%. *Dose*: 1 to 3 tablets.

Renipas Constipation Tablets (*International Chemical Research Association Ltd., London*). "A valuable cathartic and intestinal tonic, which imparts tone to the intestines, establishing there a normal physiological action, thereby preventing constipation and curing diseases arising therefrom." *Approximate Formula*:—R. Extr. Casc Sag 48, Aloini 19, Res. Podophylli 6, combined with solid and liquid ancillaries q.s. *Dose*: 2 tablets.

Renipas Powder (*International Chemical Research Association Ltd., London*). "In all cases of rheumatism, sciatica, gout, neuralgia, pains in the head (headache) or face (toothache), earache, insomnia, excess of uric acid." *Approximate Formula*:—R. Pulv. Phen. 12, Caffein. Cit. 2.5, Ac. Aceto Sal. 60, combined with solid and liquid ancillaries, Q.S. *Dose*: 1 powder.

Reudel Bath Salts (*The International Chemical Co., Ltd., London*). "For corns, callouses, aching, swelling, burning, itching, smarting, perspiration, tenderness, chilblains, gouty feet, and other foot troubles. A highly concentrated compound especially prepared to form an artificial mineral water impregnated with mineral alkali and reproducing the essential constituents found in water at the famous baths of Carlsbad, Aix les Bains and Buffalo Lithia Springs."

Rheumagic Brand Oils (*International Laboratories Ltd., London*). "Concentrated, penetrative, analgesic liniment. Stops pain and destroys the cause. Rheumatism, sciatica, arthritis, neuritis, lumbago, headaches, athletic strains, neuralgia—they all respond to its soothing curative powers." *Formula*:—Methyl Salicylate 3 parts, Ol. Eucalyptus 3 parts, Ol. Pini Dulc. 10 parts, Oleo Resin Capsici 0.6 parts, Ol. Terebinth to 100 parts.

Rheumogen (*Rheumogen Ltd., Bath*). "Takes the pain out of rheumatism, sciatica, neuritis, catarrhic colds, stiff joints and sprains. Will not blister." *Formula*:—Capsicum 10%, Acidum Salicyl. 6%, Methyl Salicyl 2%, Paraffin Molle 74%, Paraffin Dur. 8%.

Rhuaka Brand Sweet Digestive Syrup (*Rhuaka Remedies* (1923) *Ltd.*, *Halifax*). "The sunshine remedy. Will do you good. Relieves while you sleep. For acute indigestion and stomach pains. To remove causes of rheumatism." *Formula*:—Each fluid oz. cont. act. princ. of: *Amomi Semina* .01 drs., *Capsici Fruct.* .01 drs., *Rheum. Palmatum* .23 oz., *Cassia Acut. Fruct.* .012 drs., *Ex. Glycyrr. Liz. Liq.* .01 oz., *Sterculia Semina* .02 drs., *Cassia Acut. Fol.* .011 drs., *Theriac Nig.* .04 oz., *Aggr. Eupt.* .04 drs., *Trichloro-Methane* 1 min., *Marrubium Vulg.* .04 drs. *Dose*: 1 tablespoonful. Children, $\frac{1}{2}$ teaspoonful to 1 dessertspoonful.

Roberts' Croupine (*Roberts Croupine Ltd.*, *Bolton*). "For the throat, chest and lungs. For colds, coughs, croup, bronchitis, whooping cough, influenza, sore throat, ulcerated throat, difficult breathing, asthma."

Formula:—Ext. Lobel. 0.71, Ext. Grindel 0.09, Ext. Anis. Ext. Tussilag, Ext. Prun. Serot, a.a. 0.18, Sacch. Ust. 0.6, Ol. Anis. 0.1, Ac. Salicyl 0.01, Ac. Acet. (80%) 0.1, Sucrose 59.8, Aquam ad 100. *Dose*: Children, 5 drops to $1\frac{1}{2}$ teaspoonfuls, according to age; Adults, 1 to 2 teaspoonfuls.

Roboleine (*Oppenheimer, Son & Co. Ltd.*, *London*). "Renews health. Restores vigour. A reconstructive tonic food containing the essential vitamins."

Formula:—Vitamin Oil Concentrate (102,000 I.U. Vitamin A and 34,200 I.U. Vitamin D per gramme) 0.1%, Medull. Rub. 0.5%, Medull. Flav. 5.0%, Ovi vitel 0.75%, Ext. Malt to 100. *Dose*: 1 eggspoonful to 1 teaspoonful.

Roche's Embrocation (*W. Edwards & Sons, London*). "The embrocation will be found serviceable in cases of croup, bronchitis and lumbago."

Formula:—Camphora 1.125%, Ol. Carui 1.172%, Ol. Anethi 1.172%, Ol. Rosmarini 0.076%, Ol. Cajaputi 0.076%, Colour Trace, Ol. Rapii up to 100.00%.

Rooke's (Dr.) Oriental Pills (*Dr. Charles Rooke Ltd.*, *Leeds*). "A famous physician's remedy. For all liver and stomach ills."

Formula:—Ext. Gent. 22.85, Ext. Anthemidis 20.00, Pulv. Aloes Soc. 11.44, Pulv. Rad. Rhei 11.44, P. Rad. Zingib. 5.72, Podoph. Resin. .70, Pulv. Gum. Myrrh. 5.82, P. Rad. Ipecac. 4.28, P. Ext. Coloc. Co. 5.72, P. Sapo Cast. 11.44, Ol. M. Pip. .34, Ol. Anisi .34, Syr. Glucose q.s. *Dose*: 1 to 3 pills; Children, $\frac{1}{2}$ to 1 pill.

Rowland's Golden Macassar Oil (*Messrs. A. Rowland & Sons Ltd.*, *London*). "For the growth, restoration, and for beautifying the human hair. Long delays thinning and baldness." The composition of this preparation was not disclosed.

Rozalex Brand (*Rozalex Ltd.*, *Manchester*). "Keeps hands clean. Keeps hands free from cracks and chaps. Non-caustic, non-greasy, non-gritty." The composition of this preparation was not disclosed.

Ru-Mari Brand Compound (*Ru-Mari Ltd.*, *London*). "The unrivalled treatment for rheumatic diseases. Being a powerful germicidal and antacid scientific preparation."

Formula contains in aqueous solution:—Sodium Carbonate 0.81% w/v, Sodium Chloride 0.60% w/v, Sodium Sulphate 0.08% w/v, Sodium Hypochlorite 0.05% w/v, Potassium Carbonate 0.64% w/v, Calcium Oxychloride 0.04% w/v, Colour Trace. *Dose*: 1 tablespoonful.

Sal Alterata (*Wyleys Ltd.*, *Coventry*). "Uric Acid eliminant. Indicated in gout, rheumatism, sciatica, lumbago, constipation, biliousness, gravel and all diseases associated with uric acid diathesis."

Formula:—100 parts represent: Strontium Lactate 0.30, Lithium Citrate 0.15, Caffeine Citrate 0.03, Quinine Phosphate 0.06, Sodium Benzoate 0.23, Sodium Formate 0.08, Calcium Lactophosphate 0.15, Magnesium Sulphate 8.00, Sodium Sulphate 30.00, Potassium-Sodium Citro-tartrate 61.00. *Dose*: 1 teaspoonful to 1 tablespoonful.

Sal Hepatica (*Bristol-Myers Co. Ltd.*, *London*). "An effervescent balanced saline, laxative and eliminant. Tones internal functions. Maintains alkaline reserve."

Formula:—Acid. Tart. and Cit. 32.28%, Sod. Bicarb. 31.78%, Sod. Sulph. 23.83%, other constituents 12.11%. *Dose*: 1 to 2 teaspoonfuls in water.

Salinax (*British Drug Houses, London*). "Contains an aperient principle derived from the grape combined with an alkaline salt to produce a health-giving and invigorating effervescent saline draught."

Formula:—Acid. Tart. 45.92%, Sod. Bicarb. 54.08%. *Dose*: 1 or 2 teaspoonfuls in a glass of water.

Samona Brand Tablets No. 1 (*Samona Ltd.*, *London*). "No. 1 for men. Samona tablets are guaranteed to give an increased mental capacity, personal confidence and assurance, the power to think and act quickly, with the ability to carry on under pressure. A wonderful restorative of great potency."

Formula:—Bitestin Standard 2 units, Vitamin E Conc. min. $\frac{1}{10}$ th, Corp. Glandulæ Compos.

gr. $\frac{5}{8}$ th, Pulv. Cinch. Rub. gr. $\frac{1}{2}$, Tr. Iod. fr. Cod. min. $\frac{1}{10}$ th; Excipients q.s.
Dose: 1 tablet.

Samona Brand Tablets No. 2 (*Samona Ltd., London*). "No. 2 for women. Gives speedy relief in all cases of neurasthenia, nerve strain, depression, loss of vitality and general weakness." *Formula*:—P. Cinch. Rub. 35.00, Tr. Iode. Officin. Fr. Cx. 1.75, Thymus Sicc. 8.75, P. Glycyrrhizæ 17.50, Dihydroxy-phthalophene 2.75, Embryonic Subs. 8.75, Trihydroxy-œstrin 0.0105, Excipient to 100. *Dose*: 1 tablet.

Sanacine Cough Mixture (*Phosferine (Ashton Parsons) Ltd., Watford*). "For coughs and colds. The most effective remedy." *Formula*:—Extractum Marrubii 0.84%, Oleum Eucalypti 0.01%, Extractum Glycyrrhizæ 1.56%, Sp. Ment. Pip. 0.13%, Oleum Anisi 0.03%, Extractum Piper Nigrum 0.26%, Extractum Senegæ Liq. 0.13%, Saccharum Ustum 0.62%, Tincture Bryonia 0.52%, Sp. Rectificatus 0.06%, Syrupus 95.84%. *Dose*: Adults, 1 teaspoonful.

Sanatogen (*Genatosan, Loughborough*). "An unrivalled reconstituent and strength producing food for both children and adults in nervous diseases, etc." A brand of casein and sodium glycerophosphate. The composition of this preparation was not disclosed. *Dose*: 2 teaspoonfuls.

[P] **Sanderson's Cough Linctus** (*Sandersons (Chemists) Ltd., Manchester*). "For the relief and cure of coughs, bronchitis, asthma, and similar affections of the chest and lungs." *Formula*:—Anhydrous morphine .0525% w/v, Codeine .002, Tinct. Cardamon. B.P.C. '23, 2.083, Ol. Carri Ang. .0025, Ol. Cinnamonii .0025, Tinct. Persionis .2343, Chloroform .965, Spiritus Rectificatus 4.281, Colouring q.s.; Base Ad 100. *Dose*: $\frac{1}{2}$ teaspoonful; Children over 12 years, $\frac{1}{4}$ teaspoonful.

Sanderson's Throat Specific (*Sandersons (Chemists) Ltd., Manchester*). "For quinsy, sore throat, hoarseness and all throat troubles." *Formula*:—Acid. Acetic B.P. 2.250; Acid Sulph. Dil. B.P. 2.084, Capsicum Fructus .104, Scillæ 1.250, Spiritus Rectificatus .324; Base ad 100.0. *Dose*: 2 teaspoonfuls.

Sanitas (*The Sanitas Co., London*). "The antiseptic and germicide for the mouth and teeth, the sick room, cleansing cuts and wounds, personal hygiene and in all cases where a non-poisonous disinfectant is required." *Formula*:—Soluble oxidation products of terpenes 2% in aqueous solution.

Sargol (*Sargol Co. Ltd., London*). "The great specific for putting flesh on thin people, increasing weight, rounding out the figure, building up those who are run down and overworked and those suffering from nervous debility, dyspepsia, anæmia and paleness." *Formula*:—Calcium Hypophosphite .50 gr., Potassium Hypophosphite .25 gr., Sodium Hypophosphite .25 gr., Hæmoglobin .50 gr., Dried Yeast 1 gr., Zinc Phosphide .05 gr., Excipient. *Dose*: 1 or 2 tablets.

Schiffmann's (Dr. R.) Asthmador (*R. Schiffmann & Co., Los Angeles*). "For the instant relief of bronchial asthma, hay fever and spasmodic croup." *Formula*:—This powder contains 51.8% of Datura Stramon., Datura Tatula and Dat. Meteloides.

Scott's (Dr.) Brand Bilious and Liver Pills (*W. Lambert & Co. Ltd., London*). "Constipation, indigestion, loss of appetite, rheumatism, and all bilious and liver complaints." *Formula*:—Aloes Soc. 16.56%, Aloes Barb. 10.94%, Rhei 16.25%, Zingib. 13.75%, Sapo 12.5%, Scammy Res. 20%, Glycyrr. 2.5%, Excipients 7.5%, Ol. Caryoph. S.V.R. Aq. *Dose*: 1, 2, 3 or 4 pills.

Scott's Emulsion (*Scott & Bowne, London*). "A reliable remedy for pulmonary diseases, coughs, colds and general debility." *Formula*:—Ol. Morrhuæ 196 minims, Calcii and Sodii Hypophosphis 9.25 grains, Glycerini 59 minims, Pulv. Trag. 2.7 grains, Ol. Essential 5 minims, Aqua q.s. 1 oz. *Dose*: 1 tablespoonful.

Sedicyl Brand Tablets (*Veritas Drug Co. Ltd., London*). "For climacteric vasomotor disturbances." *Formula*:—Each tablet contains: $\frac{3}{16}$ gr. Phosphoric Acid salt of β -bromoethyl-trimethyl-ammonium-theobromine- β -acetate, $\frac{3}{16}$ gr. benzyol succinate. *Dose*: 1 to 2 tablets.

Sedin Liquid (*Hommel's Hæmatogen and Drug Co., London*). "Soothing for people suffering from nerves or sleeplessness. Gives a palatable drink which is not inferior to a good meat broth." The composition of this preparation was not disclosed. *Dose*: 1 teaspoonful.

Sedobrol (*Roche Products Ltd., Welwyn Garden City*). Sedative bouillon tablets. "A very appetising and palatable soup. Widely recommended as a gentle sedative which, thanks to its calming effect on the nervous system, allows sleep to come naturally at night." *Formula*:—Each tablet contains about

17 grains of sodium bromide with vegetable extractives. *Dose:* 1 or 2 tablets in boiling water.

[P1-81-87] **Sedormid Brand Sedative Tablets** (*Roche Products Ltd., Welwyn Garden City*). *Formula:*—Each tablet weighs 0.45 gm. and contains 0.25 g. Allyl-isopropyl-acetyl-urea. *Dose:* Sedative, $\frac{1}{2}$ to 1 tablet; Hypnotic, 1 to 2 tablets.

Semori (*Medical Laboratories Ltd., London*). "A foam contraceptive tablet. Non-irritant and antiseptic." *Formula:*—Ortho-oxy-pyridine Sulph. 0.5%, Kali Borotart. 32.0%, Corpus Spumans 67.5%.

Semprolin Petroleum Emulsion (*Semprolin Products Ltd., Edinburgh*). "Specially prepared by an improved process from pure Petroleum, redistilled, filtered and standardised for internal use." *Formula:*—Contains "Semprolin" Liquid Paraffin 25%. *Dose:* 1 teaspoonful.

Serocalcin (*Harwoods Laboratories, Watford*). "For the treatment and prevention of colds." *Formula:*—Each tablet contains sulphoguaiacolic plasma precipitate 0.035 gm., and excipient 0.165 gm. *Dose:* 1 to 3 tablets.

[P1] **Shadforters** (*Shadforth Prescription Service Ltd., London*). "Shadforth Prescription No. 285. Known as Bladder and Kidney Comforters, 'Pil. Kava Kava Co.', 'Dutch Drops' and 'Hollands Gin Pills.' A soothing, antiseptic and stimulant in catarrhal conditions of kidney, bladder and urinary passages." *Formula:*—Oleo Resin Kava $\frac{1}{8}$ th gr.; Cubebs $\frac{3}{8}$ th gr., Venice Turpentine $\frac{1}{2}$ gr., Copaiba Balsam $\frac{1}{2}$ gr., Dry Extract of Colchicum B.P. $\frac{1}{4}$ th gr., Gravel Nut Root $\frac{1}{2}$ gr., Podophyllin Root $\frac{1}{2}$ gr., Hexamethylene Tetramine $\frac{1}{2}$ gr., Sandal Wood Oil a trace. In one pill. *Dose:* 1 to 3 pills.

Shurzine Brand Antiseptic Ointment (*The Shurzine Pharmacy Ltd., Hastings*). "Cures: chilblains, sunburn, burns, bruises, sores, eczema, chaps, ulcerated legs, piles, psoriasis, rashes, insect bites, cuts, and all skin troubles." *Formula:*—Adep. Lanæ Anhyd. 38.83%, Paraf. Molle Flav. 36.83%, Zinci Oxid. 6.91%, Glycerol 7.67%, Phenol 1.49%, Ol. Eucalypt. 2.11%, Ol. Lavand. 2.04%, Aq. q.s.

[P1] **Silbe Brand Asthma Tablets** (*Silten Ltd., London*). "Standard asthma remedy. The remedy without harmful after-effects." *Formula:*—Ephedrine Hydrochloride 7.15%, Theobromine 14.30%, Calcium Phenylmethylbenzoiccarboxylate 28.60%, Phenylsemicarbacide 28.60%, Amylum 21.35% *w/w*. *Dose:* 1 tablet.

Silf Obesity Tablets (*The Silf Co., London*). "The safe treatment. This effective tonic reducer is prepared from pure vegetable extracts." *Formula:*—Ext. Fuci Sicc. gr. $3\frac{1}{2}$, Rheum (Rad. Rhei) gr. $\frac{3}{4}$, Ext. Casc. SAGR. gr. $\frac{1}{2}$, Aloin gr. $\frac{1}{16}$, Excipients q.s. *Dose:* 1 tablet.

Silvikrin Hair Tonic Lotion (*Drugs, Limited, London*). "Promotes the growth of healthy hair. Removes and prevents dandruff. Stops falling hair. Keeps the scalp healthy and clean." *Formula:*—Principal Constituents: 1. Pure Silvikrin (Brit. Patent 411,009); 2. Medicated Alcohol.

Silvikrin (Pure) (*Drugs Limited, London*). "The concentrated organic solution of the 14 vital elements essential for the growth of healthy hair. Pure Silvikrin should be used for severe dandruff, serious loss of hair, thinning hair, hardening of the scalp, bald patches and baldness." *Contains:* "the active principles of millions of healthy hair cells. The molecules of those cells have been split up into Tryptophane, Tyrosine, Cystine and Cysteine." Brit. Pat. No. 411,009.

[P1] **Simodine** (*Robinson, Mfg. Chemist, Blaenavon*). "The recognised treatment for asthma and bronchitis. Invaluable for shortness of breath and whooping cough." *Formula:*—Each 5 grain tablet contains *w/w* Pulv. Rad. Ipecac. 2%, Ext. Grindelia 2%, Ext. Lobelia 2%, Phenolphthalein 2%, Ext. Euphorbia 2%, Ephedrine Hyd. 10%, Pulv. Digitalis 0.06%, B.P. units of activity 2%, Pulv. Zingib. 3%, Theobrom. Sod. Sal. 2%, Pot. Iodi 10%, Pulv. Gentian, 63%. *Dose:* 1 tablet.

Simpson Brand Blood Cleanser (Iodised) (*J. W. Simpson (Chemist) Ltd., London*). "Highly recommended for clearing the blood of impurities, thereby removing the cause of skin eruption, eczema, boils, ulcerated legs, rheumatism, lumbago, and allied complaints." *Formula:*—Iodide of Potassium 1.09%, Chloride of Ammonium 0.76%, Trichloromethane 0.24%, Sodii Nucleas 0.21%, Sodium Salicylate 1.4%, Potass. Bicarb. 40%, Inf. of Gentian Conc. 0.11%, Spirit Vini Rectificatus 0.24%, Solution Sacch. Ust 0.5%, Aq. Destillata 94.45%. *Dose:* 1 tablespoonful; Children, 1 teaspoonful to 1 dessertspoonful.

Simpson Iodine Ointment (*J. W. Simpson (Chemist) Ltd., London*). "For cuts, chilblains, insect bites, sores and skin eruptions of all kinds. Reduces bunion inflammation; stops rheumatic, neuritis and lumbago pains when massaged in." *Formula*:—Iodine 5%, Ol. Arach. 15%, Methylis Salicylas 5%, Ol. Cajaput 2%, Adeps. Lan. 20%, Paraff. Moll. Flav. 53%.

Simpson Iodolok Iodine Locket Diffuser (*J. W. Simpson (Chemist) Ltd., London*). "For perfect health iodine is as essential as all the vitamins. Increases your resistance to flu, colds, and all other germ diseases." The composition was not disclosed.

Simpson Seaweed and Celery Tablets (*J. W. Simpson (Chemist) Ltd., London*). *Formula*:—Ext. Fucus $\frac{1}{2}$ gr., Sodii Salicyl. $\frac{1}{2}$ gr., Phenolphthalein $\frac{1}{2}$ gr., Hydrarg. Subchlor. $\frac{1}{2}$ gr., Pulv. Celery $\frac{1}{2}$ gr., Saccharinum $\frac{1}{100}$ gr., Choc. base up to 6 gr. *Dose*: 2 tablets.

[P1] **Singha (Dr.) Brand Asthma Tablets** (*The Dr. Singha Co. Ltd., Caernarvon*). "For the treatment of asthma, bronchitis, difficulty in breathing, choking paroxysms." *Formula*:—Trimethylxanthine gr. $\frac{1}{2}$, P. Grindel gr. 1, Emet. Hydrochlor. B.P. gr. $\frac{1}{100}$, Lobel B.P. gr. $\frac{1}{2}$, Ext. Euphorbia Pilulif gr. $\frac{1}{2}$, Ephed. Hydrochlor. B.P. gr. $\frac{1}{10}$, Amylum gr. $\frac{1}{2}$, Lactose ad gr. 5. *Dose*: 1 tablet.

[P1] **Singleton's Eye Ointment** (*Stephen Green Ltd., London*). "For many eye troubles and diseases. The standard remedy which has outlived the centuries." *Formula*:—Red Mercuric Oxid. 5.45% w/w.

Sloan's Liniment (*Dr. Earl S. Sloan Ltd., London*). "Kills pain. Rheumatism, lumbago, sciatica, neuralgia, stiff neck, neuritis, backache, bruises, sprains, strains, insect and mosquito bites." *Formula*:—Ol. Camph. (Synthetic) 20%, Liq. Ammon. Fort. 0.03%, Ol. Pini Sylves. 3%, Methyl Salicyl. 3.5%, Ol. Terebinth 39.9%, Paraffin soluble constituents of 6% Capsic.

Smedley's Paste (*Hirst, Brooke & Hirst, Leeds*). "An external application for the relief of rheumatism, lumbago, sciatica, bronchitis, sore throat, neuralgia, etc., unbroken chilblains." *Formula*:—Consists of an absorbent fatty base containing 36% w/w of Capsicums.

Songo Brand (*Vandré Ltd., Glasgow*). "Ends all travel sickness. Prevents seasickness, bus, train, air and motor sickness." *Formula*:—Trichlor-Tertiary-Butyl Alcohol 89.022%, Tri-Methyl-Xanthine 10.976%, Crocus Sativus 0.002%.

Sotol Antiseptic Mouth Wash Tablets (*The Western Dental Mfg. Co. Ltd., London*). *Formula*:—Sodii Bicarb. 4 $\frac{1}{2}$ gr., Acid Tart. 3 $\frac{3}{4}$ gr., Sodii Boras 1 $\frac{1}{2}$ gr., Thymol $\frac{1}{100}$ gr., Menthol $\frac{1}{100}$ gr., Gluside and Flavouring Essences q.s.

Spasmolyth Brand Powders (*Warwick Pharmacals Ltd., London*). "The new internal treatment. The most satisfactory solution to the Problem of Asthma." *Formula*:—Phenapyrine-Caffeine (Condensation product of Phenazone 0.275, Phenacetin 0.125, and Caffeine 0.100) 0.50, Phenazone 0.45, Iodopyrine 0.03, Ext. Grindelia 0.01, Cereus Grandiflorus 0.01. *Dose*: 1 powder.

Sphagnol Lotion 10% (*Peat Products (Sphagnol) Ltd., London*). "For use where a non-greasy skin dressing is required." *Formula*:—Pix Liquida 9% w/v, Xylenols 1% w/v.

St. Jacob's Brand Liniment (*International Chemical Co., London*). "An external remedy for pain." *Formula*:—Camphora 7.00%, Mezereum 2.10%, Phenol 0.584%, Ol. Origanum 3.40%, Ol. Terebinth. 60.51%, Ether 9.40%, Spirit. Ind. 17.00%, Colour 0.006%.

St. James' Balm (*Medico-Biological Laboratories Ltd., London*). "Antivirus ointment for every skin trouble. Removes the cause of skin troubles and rapidly heals damaged tissues." *Formula*:—"Anti-virus of the germs of skin infections, streptococci, staphylococci, B. Pyocyaneus, 12.50, Paraff. Moll. Flav. 7.25, Ichthyolammon. 1.25, Zinc. Oxide 20.00, Paraff. Liq. Alb. 14.50, Adeps Lanæ Anhyd. 42.00, Ol. Theobrom 2.50."

Steedman's Soothing Powders (*John Steedman & Co., London*). "For children cutting their teeth." *Formula*:—Hydrarg. Subchlor. 26.7, Ipecac. Pulverat. 2.2, Sucros. 17.8, Amylum 53.3.

Stemcol (*Stemco Ltd., London*). "For coughs." *Formula*:—Acet. Scillæ B.P. 1914 1.9%, Ext. Ipecac. Liq. 0.14%, Tinct. Camph. Co. B.P. 1914 sine Opio. 3.1%, Mel. Depur. 2.5%, Syr. Pic. Liq. 2.5%, Syr. Tolu. 2.5%, Glycer. 3.4%, Acac. et Trag. 1.9%, Paraff. Liq. 25%, Aq. Dest. ad 100%. *Dose*: Adults, 1 dessertspoonful; Children, $\frac{1}{2}$ to 1 teaspoonful.

Stemco Brand Kaolin Poultice (*Stemco Ltd., London*). "For inflammation and congestion." *Formula*:—Kaolin 52.7%, Boric Acid. 4.5%, Thymol

0.05%, Methyl Salicylate 0.20%, Oil of Peppermint 0.05%, Sodium Lactate (70%) 42.5%.

Storaxol (*Parke, Davis & Co., London*). "An antipruritic, parasiticide and antiphlogistic ointment." *Formula*:—Each ounce (28 gm.) represents Storax 9 grains (0.58 gm.), Resorcin 2 grains (0.13 gm.), Menthol 2 grains (0.13 gm.), Camphor 9 grains (0.58 gm.), Phenol (Carbolic Acid) 22 grains (1.42 gm.), Precipitated Sulphur 87 grains (5.63 gm.), in a combined Lanolin and Petrolatum base.

Sucrets Lozenges (*Sharpe & Dohme Ltd., London*). "Tested for germicidal activity. Produces a marked soothing effect on the irritated mucous surface of the mouth and throat." *Formula*:—Each lozenge contains approx. 2.5 mg. Hexylresorcinol.

Sulphaqua (*The S.P. Charges Co., St. Helens*). "A convenient preparation for the production of nascent sulphur in the ordinary domestic bath. For the treatment of skin diseases, gout, rheumatism, etc." *Formula*:—Approximate composition: Pulv. Sodii Thiosulphas Exsicc. 44%, Pulv. Sodii Bisulphas 56%.

Sulpholine Lotion (*John Pepper & Co. Ltd., London*). "For clearing the skin from eruptions, spots, redness, roughness, freckles, tan and pimples. For eczema and other obstinate skin diseases." *Formula*:—Sulphur Præcip. 4.0%, Zinc. Oxid. 2.0%, Glycer. 7.8%.

Surama Medicated Cigarettes (*Royal Court Mfg. Co., London*). "Cure colds, coughs, and nasal catarrh. Relieve asthma, bronchitis and hay fever." *Formula*:—P. Cort. Cascarilla 1.5%, P. Fruct. Cubebæ 1.5%, P. Gum Benzoin 1.5%, Fol. Stramonii 45%, Fol. Rosæ 47.5%, Ol. Eucalyptii Glob. 1.5%, Menthol 0.5%, Ol. Pini Pumil 1.0%.

Sure Shield Brand Fruit Laxatives (*Thos. Guest & Co. Ltd., Manchester*). "Both palatable and effective. Better than Castor Oil." *Formula*:—Each tablet contains Phenolphthalein 1.381 grains, Succus Rubus Idæus 1.010 grains; With Sacchar. Alb. and Lubricant Base. *Dose*: Adults, 1 tablet; Children $\frac{1}{2}$ to 1 tablet.

Sure Shield Brand Iodised Throat Tablets (*Thos. Guest & Co. Ltd., Manchester*). "For the voice, mouth and throat." *Formula*:—Tr. Iodine M. $\frac{1}{4}$, Ac. Carbolic M. $\frac{1}{4}$, Menthol Gr. $\frac{1}{4}$, Ac. Citric Gr. $\frac{1}{2}$, Ol. Gautheria M. $\frac{1}{16}$, Sugar q.s.

Sure Shield Brand Maclean's Stomach and Digestive Powder (*Thos. Guest & Co. Ltd., Manchester*). "Invaluable in all cases of indigestion, gastritis, ulcerated stomach, flatulence, heartburn, dyspepsia, etc." *Formula*:—Bismuth Oxycarb. 9.99 parts, Sodii Bicarb. 18.18 parts, Magnes. Carb. Pond. 35.915 parts, Calcii Carbonatis 35.915 parts.

Sure Shield Brand Tonic Tablets (*Thos. Guest & Co. Ltd., Manchester*). "For all disorders caused by exhaustion of the nervous system. An invaluable remedy for anæmia, general depression, debility, sleeplessness, nervous breakdown, muscular fatigue and loss of energy." *Formula*:—Ferri Phosph. Sacch. 7.5, Ferri Carb. Sacch. 50.5, Calcii Hypophosph. 5.5, Sodæ Sulphocarb. 7.5, Quinine Sulph. 1.5, Ext. Cascara Sag. 1.5, Sacchar. Alb. c. Amylum 23.0, Lubricant 3.0. *Dose*: 1 or 2 tablets.

Surgaseptic (*Wands Ltd., Leicester*). "Trichlorophenylmethyliodosalicyl in water. The perfect treatment for sore throats, tonsilitis, bad breath, burns, cuts, abrasions." *Formula*:—Contains halogenized phenolic compounds made from the following ingredients:—Phenol 0.63%, Chlorine 0.22%, Salicylic Acid 0.06%, Iodine 0.15%.

Sustenoid Brand Yeast Tablets (*Thompson & Capper Wholesale Ltd., Liverpool*). "To be taken for indigestion, pimples, boils, skin blemishes, poor condition of the blood, constipation, neuritis, bad condition of the nerves, poor general health causing faintness, headaches, dizziness, sleeplessness, depression." *Formula*:—100% pure yeast.

Suthers' Chrono-Thermal Composition Essence (*Wm. Suthers, Manchester*). "For coughs and colds." *Formula*:—Capsic. 0.625%, Cinnam. 1.3125%, Alcohol (60%) 13.33%, Ol. Caryoph. 0.14%, Pini Canad. 0.75%, Hamatox. 0.33%, Pot. Bicarb. 0.28%, Myric. 2.5%, Aqua ad 100%. *Dose*: 1 or 2 teaspoonfuls in a cupful of hot water.

Sweeting's Toothache Elixir (*Barclay & Sons Ltd., London*). "The finest nervine tonic ever discovered. Is a certain cure for the toothache." *Formula*:—Tinct. Zingib. Fort. 1.2%, Tinct. Chirar. 7.5%, Ol. Pœnic. 0.2%, Tinct. Cinchon. to 100%. *Dose*: 1 or 2 teaspoonfuls.

[P1] Tabloid Brand Laxative Vegetable (*Burroughs Wellcome & Co. London*). *Formula*:—Ext. Coloc. Comp. B.P. 1914, gr. 1; (65 mgm.), Ext.

Jalapæ gr. $\frac{1}{2}$ (32 mgm.), Podoph. Resinæ gr. $\frac{1}{2}$ (16 mgm.), Leptandrin gr. $\frac{1}{2}$ (32 mgm.), Ext. Hyoscy. Vir. P.B. 1898, gr. $\frac{1}{2}$ (16 mgm.), Ext. Taraxaci P.B. 1914, gr. $\frac{1}{2}$ (16 mgm.), Ol. Ment. Pip. q.s. Dose: 1 to 3 tablets.

Tamar Indian Brand Laxative Fruit Lozenges (*Tamar Indian, Hampton*). "For constipation and its attendant maladies, such as hæmorrhoids, headache, congestion of the liver, want of appetite, disorder in the stomach and intestines." *Formula*:—Tamarind 55.0, Senna Fruit 23.0%, with chocolate coating. Dose: 1 lozenge.

Taxol Brand Tablets (*Continental Laboratories, London*). "Physiological treatment of constipation." *Formula*:—Intestinal gland Ext. 0.05 gr., Biliary Extract 0.10 gr., Agar Agar 0.05 gr., Lactic Ferment. 0.05 gr. Dose: 2 tablets.

Taylor's Seven Oils (*H. Bellringer, Manchester*). "For muscular rheumatism, sprains, sore throats, lumbago, sciatica, etc." *Formula*:—Capsicum 1.8%, Ol. Terebinth. 64.0%, Ol. Camph. Continent. 4.0%, Ol. Succini Rect. 2.00%, Camphora .2%, Menthol .6%, CHCOOH 33% 4.0%, Saponis .4%, Ovum. Subst. 1.6%, H₂O ad 100.0%.

T.C.P. (British Alkaloids Ltd., London). "The perfect antiseptic. For external and internal use. Non-toxic, non-caustic, analgesic, antithermic, endosmotic, keroplastic, non-hæmostatic, stable." *Formula*:—A solution of halogenated phenolic bodies in water made from the following ingredients—Chlorine 0.2%, Iodine 0.11%, Phenol 0.63%, Salicylic Acid 0.045%, Bromine a minute trace.

T.C.P. Bronchial Antidote (*British Alkaloids Ltd., London*). "For coughs, bronchitis, asthma, whooping cough and similar bronchial affections." *Formula*:—T.C.P. 6.7%, Iodised Sodium Salicylate 0.5%, Sodium Salicylate 1.5%, Sugar 22.3%, Sodium Bicarbonate 1.7%, Ext. Malti 14%, Liq. Pruni Virg. 2.7%, T.C.P. = Chlorine 0.2%, Iodine 0.11%, Phenol 0.63%, Salicylic Acid 0.045%, Bromine a minute trace.

T.C.P. Medium Ointment (*British Alkaloids Ltd., London*). "Recommended for acne, impetigo, sprains, bruises and for wound dressings, etc." *Formula*:—T.C.P. 17.0, Iodum 0.6, Methyl Sal. 3.0, Sulphur 4.0, Camphor 3.5, Ac. Salicyl. 1.0, Ac. Boric. 3.0, Glycerine 5.0, Colloid. Kaolin 22.65, Petroleum Excip. 200.0. T.C.P. = Chlorine 0.2%, Iodine 0.11%, Phenol 0.63%, Salicylic Acid 0.045%, Bromine a minute trace.

T.C.P. Throat Pastilles (*British Alkaloids Ltd., London*). "Unsurpassed antiseptic properties. Soothing and pleasant." *Formula*:—T.C.P. 11.6% (Trichlorophenylmethylidodisalicyl in H₂O), Glycerine 1.25%, made up on a pure black currant fruit base.

[P1] **Teasdale's Chlorodyne** (*The Teasdale Chlorodyne Co., Huddersfield*). "For the relief of coughs, colds, influenza, bronchitis, hysteria, sea-sickness, diarrhoea, colic, cramp, spasms, etc." *Formula*:—Tinct. Capsic. 7% v/v, Ether Meth. 3% v/v, Ol. Ment. Pip. 0.138% v/v, Theriaca 62% v/v, Chloroformum 9% v/v, Morph. Hydrochlor. R.P. 0.174% w/v, Codeinæ Hydrochloridum B.P.C. 0.43% w/v, Hydrocyanic Acid (HCN) 0.0017% w/w, Aqua ad 100.

Teddylax (*Sister Laura's Food Co. Ltd., Glasgow*). "Chocolate laxative sweets for children and adults." *Formula*:—Chocolate 98%, Phenolphthalein 2%.

Tersalts (*Reid & Donald, Perth*). "A safe remedy for rheumatic affections, sciatica, unhealthy conditions of the skin, biliousness, constipation, disorders of the kidneys, liver and stomach." *Formula*:—A compound of Soda Sulph., Magnes. Sulph., Potass. Sulph., Potass. Tart., of great purity prepared by a scientific process. Dose: $\frac{1}{2}$ to 1 teaspoonful.

Tetmal (*Wright, Layman & Umney Ltd., London*). "For headache, neuralgia, toothache and all women's ailments due to nerve suffering." *Formula*:—Caffein 5.62, Phenazon 33.75, Phenacetin 37.50, (excipient) 1.00, P. Amyli 22.13. Dose: 1 tablet.

Thermogen Medicated Wadding (*Thermogen Co., Ltd., Hayward's Heath*). "For rheumatism, neuralgia, bronchitis, lumbago, neuritis, sciatica, chest colds, etc." *Formula*:—Impregnated with 2.86% of Oleoresin of Capsicum and 0.03% of Methyl Salicylate.

Thermogene Vapour Rub (*Thermogene Co., Ltd., Hayward's Heath*). "For head colds, chest colds, nasal catarrh, sore throat, neuralgia, rheumatic and similar aches and pains." *Formula*:—Camphor 3.00, Menthol 2.00, Oleores, Capsic 0.02, Methyl. Salicylat. 15.00, Ol. Terebinthinæ 5.00, Ol. Camph. Essent. 8.00, Ol. Caryophylli 2.00, Cineol 2.00, Combined with a compound Lanolin-Wax base to make 100.

Thompson's (Dr.) Dandelion Coffee (*Potter & Clarke Ltd., London*). "Is a natural beverage without injurious effects. Exercises a stimulating influence over the whole system, helping the liver and kidneys to do their work and keeping the bowels in a healthy condition. Contains neither chicory nor ordinary coffee, but is made from pure English dandelion roots."

Tiz (*International Chemical Co. Ltd., London*). "For tender feet. A delightful toilet tablet for the relief of tired perspiring and tender feet." The composition of this preparation was not disclosed.

Towle's Brand Pills (*E. T. Towle & Co. Ltd., Nottingham*). "Pennyroyal and Steel Pills" *Formula*:—P. Fe. Sulph. Ex. 13%, P. Rhei 16%, P. Aloes Barb. 16%, P. Capsici 6%, Ext. Gent. 40%, Ol. Pulegii 0.5%. *Dose*: 2 pills.

[P1] **Towle's Chlorodyne** (*A. P. Towle & Son, Stockport*). "Invaluable for the immediate relief of coughs, colds, asthma, bronchitis, neuralgia, ague, diarrhoea, dysentery, cholera, cramp and seasickness." *Formula*:—Chloroform B.P. 20% *v/v*, Morphia Mur. 144% *w/v*, A. Hydrocyan. Dil. B.P. 0.06% *v/v*, Ol. Menth. Pip. B.P. 577%, Ol. Resin Capsici B.P.C. 0.008% *w/v*. *Dose*: Adults, 5 to 20 drops; Children, 1 to 4 drops.

Towle's Pink Pills (*E. T. Towle & Co. Ltd., Nottingham*). "For obstinate cases of suppression." *Formula*:—P. Aloes Barb. 41.791%, P. Myrrh. 14.926%, F.E. Sulph. Ex. 22.388%, Croci Sativæ 1.866%, Ol. Pulegii 747%, Ol. Rhue 747%. *Dose*: 2 pills.

[P1] **Treutabs** (*Camden Chemical Co., London*). "Have proved excellent for headache, toothache, neuralgia, tabes and attacks of a colic nature. Also render excellent service in cases of insomnia and excitement, at the beginning of and during convalescence of all infectious diseases. The effect is likewise most prompt in dysmenorrhœa." *Formula*:—Acid. Acetylsalicyl. 56%, Phenacet. 8%, Codein Phosph. 0.8%, Sod. Chloras 3.2%, Mag. Chlorid. 0.3%, Calc. Carb. 0.4%, Mag. Carb. Lev. 0.08%, Sod. Sulph. 0.02%, Excipients 31.2%. *Dose*: 1 tablet.

Triad Cherry Malt and Oil (*John Bell, Hills & Lucas Ltd., London*). "With improved Parrish's Food. A modern combination of well-tryed remedies. Is highly recommended for growing children, for body building, anæmia, rickets and for the prevention of winter ailments." *Formula*:—Active Constituents: Pot. Phos. 0.03%, Calc. Phosph. 0.12%, Ferr. (Colloid) 0.1%, Ol. Morr. 14.4%, Ext. Malt 82%. *Dose*: 1 tablespoonful; Children, 1 teaspoonful to 1 dessertspoonful.

Trilax Tablets (*The manufacturer's name is not given on the container*). "For constipation." *Formula of each tablet*:—Ext. Cascaræ Gr. 1½, Phenolphthalein, Gr. ¼ Aloin Gr. ¼. *Dose*: 1, 2 or 3 tablets.

Tubelette Brand Menthol and Wintergreen Cream (*Anglo-American Pharmaceutical Company Ltd., Croydon*). "For external use in rheumatism, lumbago, neuritis, sciatica, muscular stiffness and neuralgia." *Formula*:—Composition percentage: Capsicine 0.0250, Thymol 0.0125, Eucalyptus 0.0188, Menthol 4.0000, Methyl Salicylat. 13.9437, Mucil. Chondrus 82.0000.

Tudor Williams' Brand Balsam of Honey (*Dr. Tudor Williams, Aberdare*). "Invaluable in all cases of coughs, colds, croup, whooping cough, fevers, measles, chicken-pox, etc." *Formula*:—Honey 10.410%, Chlorof. 0.156%, Ol. Menth. Pip. 0.078%, Liq. Pot. Hydrox. 0.065%, Ether Meth. 0.156%, Tinct. Ipecac. 5.208%, Ammon. Carb. 0.52%, Spt. Vini Rect. 0.52%, Ol. Anis. 0.065%, Pot. Brom. 0.52%, Ol. Limonis Deterpenat. 0.078%, Flor. Camph. 0.026%, Colour *q.s.*, Syrup Simp. to 100%.

[P1] **Tussicon Pectoral Cough Elixir** (*Lewis & Burrows Ltd., London*). "A speedy effectual and pleasant remedy for coughs, colds, bronchitis, hoarseness and all affections of the chest, throat and lungs." *Formula*:—Syr. Scillæ B.P. 14.37-50, Sucrose 13.50, Vin Ipecac. 6.00, Chlorof. B.P. 0.50, Alcoh. 90% 2.31, Ol. Menth. Pip. 0.008, Ac. Sulph. Dil. 6.00, Morph. Hydrochlor. *w/v* 0.11, Acid. Hydrochlor. dil. 0.19. *Dose*: 15 drops to 1 small teaspoonful.

[P2] **Ulceratum Brand Ointment** (*North & Roper, Northampton*). "The healing touch, for ulcerated legs. An ointment for the relief and cure of ulcerated legs, eczema, psoriasis, itching piles, and all skin troubles." *Formula*:—Methyl. Sal. 1.63%, Zinc. Oxid. 6.68%, Plumbi Carb. 4.45%, Phenol 4% *w/v*, Paraff. Molle 57.67%, Adep. Lanæ Anhyd. 11.12%, Paraff. Durum 14.45%.

Union Jack Paste (*The manufacturer's name is not given on the container*). *Formula*:—Ac. Salicyl. 12.5, Ung. Coloph. ad 100.

Urace Brand Rheumatism Tablets (*Newbury & Phillips Ltd., London*). "A scientific preparation for the cure of rheumatism, gout, sciatica, lumbago,

stone, gravel, and all other diseases arising from excess uric acid." *Formula*:—Each tablet contains Acetylsalicylic Acid 0.13 Gm., Guaiaci Res. 0.064 Gm., Quin. Bisulph. 0.032 Gm., Amylum 0.016 Gm., Saccharum 0.016 Gm., *Dose*: 1 tablet.

Uricure Tablets (*The maker's name is not given on the container*). "The speediest and most effective of all remedies for rheumatism, gout, lumbago, and sciatica." *Formula*:—Ac. Acetylsal. gr. 4, Aloin gr. $\frac{25}{100}$, Methyl Sal. q.s. *Dose*: 2 tablets.

Urillac Liniment (*The Urillac Co., London*). "A speedy remedy for sore throats, bronchitis, rheumatism, lumbago, sprains, etc. All kinds of massage." *Formula*:—Ol. Camph. Essent., Ol. Succini, Ol. Amygdalæ, of each equal parts.

Urillac Tablets (*The Urillac Co., London*). *Formula*:—Acidum Acetylsalicylicum gr. 2.75, Phenacetinum gr. 1.0, Sulphur Sublimatum gr. 0.125, Caffeinæ Benzoas gr. 0.5, Aloinum gr. 0.022, Amylum gr. 1.5, Acacia gr. 0.25, Erythrosina gr. 0.0004, Tartrazina gr. 0.00004. *Dose*: 2 tablets; Children, 1 tablet.

Urodonal Brand (*Chatelain's Laboratories. Agents: Spencer & Co., London*). "Arthritism, rheumatism, arterio-sclerosis, obesity, renal and biliary lithiasis, gout, gravel, pains and acidity." *Formula*:—Hexamine 4.35%, Piperazine Tart. 2%, Quinac Acid 14%, Theobromine 0.6%, Lith. Carb. 25%, Sod. Bicarb. 52.5%, Acid Tart. 31.25%, Sod. Phosph. Exsicc. 5.62%, Acid Cit. 1.87%, Sacch. Alb. 3.46%, Sod. Cit. 3%.

Valda Pastilles (*Pastilval Ltd., London*). "The best for cough, cold, bronchitis, influenza, sore throat, hoarseness, asthma, etc." *Formula*:—Menthol 0.715%, Eucalyptol 0.033%, Ol. Menth. Pip. 0.011%, Terpeneol 0.0025%, Thymol 0.0025%, Pastille base ad 100.

Valkasa Nerve Food (*James Woolley, Sons & Co. Ltd., Manchester*). "It may be given with confidence in cases of nervous debility, impaired digestion, anæmia, and in wasting diseases. Also in all conditions resulting from defective nutrition such as rickets, etc." The composition of this preparation was not disclosed. *Dose*: 2 teaspoonfuls; Children, 1 teaspoonful.

Vapex Inhalant (*Thos. Kerfoot & Co. Ltd., Vale of Bardsley*). "For the prevention or relief of colds, catarrh, influenza, etc." *Formula*:—Menthol 17.500, Linalyl Acetate 0.468, Ol. Eucalypt. 4.687, Ol. Lavand. 4.687, Borneyl Acetate 0.416, Oil Camph. Essent. 1.500, Alcohol (I.M.S.) 70.742.

[P1] **Vapo-Cresolene** (*Vapo-Cresolene Co., New York: Allen & Hanburys, London*). "A vapor treatment for whooping cough, spasmodic croup, bronchial asthma, coughs, nasal colds, bronchitis, influenza." *Formula*:—Contains 97% w/v cresols.

Varicones (*Thompson & Capper Wholesale Ltd., Liverpool*). "Remedy for piles." *Formula*:—Hamamelidini 6.11%, Ol. Theobrom ad 100.

Vaseline Brand Borated Petroleum Jelly (*Chesebrough Manufacturing Co. Ltd., London*). "A valuable mild emollient for head colds, minor cuts, abrasions, burns, simple irritation of the eyelids and for minor skin irritations." *Formula*:—90% White Petroleum Jelly, 10% Boracic Acid.

Vaseline Brand Capsicum Petroleum Jelly (*Chesebrough Manufacturing Co. Ltd., London*). "A counter irritant for muscular aches and pains and superficial congestion." *Formula*:—Petroleum Jelly in combination with 1½% Oleo Resin Capsicum.

Vaseline Brand Carbolated Petroleum Jelly (*Chesebrough Manufacturing Co. Ltd., London*). "A valuable dressing for minor cuts, abrasions and burns." *Formula*:—98½% Petroleum Jelly, 1½% Carbolic Acid.

Vaseline Hair Tonic (*Chesebrough Manufacturing Co. Ltd., London*). "A liquid preparation for preserving and restoring the strength and beauty of the hair. Ends dry scalp—the cause of dandruff, scurf and falling hair." The composition of this preparation was not disclosed.

Vaughan's Bronchial Cure (*Kiloh & Co. Ltd., Cork*). "A marvellous cure for asthma, hay fever, bronchitis, loss of voice, whooping cough and consumption. Also purifies the air." A powder to be burnt and the fumes inhaled. The composition of this preparation was not disclosed.

[P1] **Veganin Tablets** (*William R. Warner & Co. Ltd., London*). "Influenza, dysmenorrhœa, migraine, neuralgia, analgesic, antipyretic." *Formula*:—Each tablet 11.8 grns., contains w/w: Acid Acetylsalicyl. 32.68%, Phenacet. 32.68%, Codeine 0.99%, Excipient ad 100%. *Dose*: 1 to 2 tablets.

Vegetex Brand Tablets (*Modern Health Products Ltd., London*). "The alkali treatment for rheumatism and kindred ailments." *Formula*:—Watercress, dried and powdered, 35%; Celery, dried and powdered, 30%; Horseradish.

dried and powdered, 15%; Parsley, dried and powdered, 10%; Lettuce, dried and powdered, 5%; Mint, dried and powdered, 5%. *Dose:* 1 to 4 tablets.

[P1-87] **Veinotrope (Formula F)** (*Continental Laboratories, London*). *Formula:*—Parathyroid 0.001 gr., Ovary 0.025 gr., Suprarenal 0.025 gr., Pancreas 0.100 gr., Posterior Pituitary 0.001 gr., Ext. of Horse Chestnut 0.005 gr., Ext. of Hamamelis Virg. 0.010 gr., Nux Vomica 0.005 gr., Excipient q.s. to 0.35 gr.

Velocium (*The manufacturer's name is not given on the container*). "The germ killing treatment. For colds, catarrh, asthma and hay fever." *Formula:*—Quinine Sulphas B.P. 47.963, Phenacetinum B.P. 10.5, Extractum Longifolia 3.5, Hydrargyri Subchloridum 2.1, Extractum Aloes 0.7, Podophylli Resina B.P. 0.7, Excipient 34.535. *Dose:* 2 tablets.

Velox Rheumatic Tablets (Vio Brand) (*Hedges Ltd., Birmingham*). "Quickly relieves rheumatism and all uric acid complaints." *Formula:*—Mono-aceticacidester of Salicylicacid 85.5, Lactose 1.75, Maranta Arun 12.75, Colura q.s. *Dose:* 2 tablets.

Veno's Brand Nasal Tablets (Veno Drug Co. Ltd., Manchester). "For all cases of nasal catarrh and hay fever." *Formula:*—Sodii Chlor. 66.17, Sodii Sulph. 18.02, Sodii Phosph. 3.02, Pot. Chlor. 5.01, Pot. Sulph. 3.02, Pot. Phosph. 4.01, Menthol 0.75. "One tablet dissolved in two tablespoonfuls of lukewarm water makes a solution of about equal specific gravity with blood serum. Spray this solution into the nasal passages once daily."

Veno's Brand Seaweed Tonic (Veno Drug Co. Ltd., Manchester). "The family remedy for complaints of the stomach, liver, kidneys and blood." *Formula:*—Ext. Cas. Sag. Liq. 12.781, Glycerol 11.143, Tinct. Pod. Res. 0.420, Sodii Hypophos. 1.005, Ext. Chionanthus Vir. 1.497, Ext. Fucus Ves. 1.556, Trichlor-methane 0.376, Aq. Destill. 71.22. *Dose:* 1 teaspoonful; Children, $\frac{1}{2}$ teaspoonful.

Veno's Lightning Cough Cure (Veno Drug Co. Ltd., Manchester). "The ideal family remedy. It should be used in cases of coughs, colds, bronchitis, sore throat, hoarseness, bronchial asthma, whooping cough, influenzal and catarrhal colds." *Formula:*—Ext. Ipecac. Liq. 0.40%, Ext. Scillæ Liq. 0.32%, Ext. Grindelia Liq. 0.90%, Chloroformum 0.70%, Tr. Capsici 0.10%, Sodii Benzoas 0.20%, Ammonii Carbonas 0.60%, Ammonii Chloridum 0.20%, Camphora 0.02%, Syrupus 48.69%, Ol. Anisi 0.04%, Aqua 47.83%. *Dose:* 2 teaspoonfuls; Children, 1 teaspoonful; Infants, 5 to 10 drops.

Veno's Liniment (Veno Drug Co. Ltd., Manchester). "Will be found beneficial in all cases of joint and muscular rheumatism, lumbago, stiff joints, sciatica, muscular weakness, pains in the chest and back, etc." *Formula:*—Ol. Terebinth 66.470%, Ol. Dulcis 22.290%, Camphora 0.964%, Ol. Cassiæ 0.482%, Ol. Eucalypti 4.460%, Chloroform 0.964%, Methyl Salicylas 4.350%, Rubrum Scarlat. 0.020%.

Veracolate Brand Tablets (W. R. Warner & Co., London). "A true cholagogue. Indications: Functional insufficiency of the liver and gall-bladder. Prophylaxis of gallstone diathesis and relief of constipation due to biliary insufficiency." *Formula:*—Sodium glycocholate 0.4 grain, Sodium Taurocholate 0.67 grain, Extract of Cascara Sagrada 1 grain, Phenolphthalein 0.5 grain, Oleoresin of Capsicum 0.05 grain. *Dose:* 1 or 2 tablets.

Vick Brand Vapour Rub (Vick Chemical Co. Distributors: J. C. Gambles & Co. Ltd., London). "A vaporising ointment for certain forms of local inflammation and congestion." *Formula:*—Menthol. 2.470, Camph. 4.608, Ol. Terebinth. 4.751, Thymol 0.094, Ol. Myrist. 0.486, Ol. Eucalyp. 1.355, Ol. Fol. Cedri 0.446, Basis ad 100.0.

[P2] **Victor's (Dr.) Ointment (J. W. Douglas Ltd., London)**. "The finest cure for all wounds, ulcers and skin diseases." *Revised war formula:*—Adeps 50 grammes, Cera alb. 75 grammes, Paraff. Molle 1620 grammes, Phenol 30 grammes, Zinci Oxid 150 grammes, Adeps Lanæ 50 grammes, Paraff. Dur. 100 grammes.

Vikelp Brand Mineral-Vitamin Tablets (Health Products Laboratories, London). "Gives new energy and vigour, corrects blood and gland deficiencies, builds strong bodies." *Formula:*—Macrocystis Pyrifera 37.50%, Calcii Phosphas (Mono Acidus) 25.00%, Ferri et Ammonii Citras 4.375%, Cupri Sulphas 0.037%, Vitamin B₁ 15 international units, Gummi Acaciæ 1.562%, Shellac 3.125%, Ferri Oxid. 1.562%, Terra Umbra 3.125%, Magnesii Silicas 23.714%. *Dose:* 3 tablets.

Vik-Wik Brand Liniment (Vik-Wik Laboratories, Rayner's Lane). "The universal pain killer for rheumatism, etc." *Formula:*—Pip. Nig. 70.87 g.,

Sem. Sinapis 70·87 Gm., Rad. Zingib. 28·850 Gm., Flor. Anthem. 42·53 Gm., Ol Citron. 2·75 Mil., Liq. Virid. 2·5 Mil., Ind. M. Spt. 4·5459L.

Vio-Ray Malt (*Burgoyne Burbridges & Co. Ltd., London*). "The perfect vitamin food. A palatable preparation of Malt and Oil containing vitamin D produced by violet ray irradiation, augmented with concentrates of vitamins A, B₁ and B₂, and concentrated orange juice." *Dose*: Children 1 to 2 teaspoonfuls; Adults, 1 to 2 dessertspoonfuls.

Viosulfal (*Viosulfal Ltd., London*). "Soluble assimilable sulphur. For the treatment of fibrositis, muscular rheumatism, arthritis, neuritis, gout, lumbago, sciatica." *Formula*:—Sulphur Sublim. 3%, Iod. 0·04%, Amylum 0·45%, Sucrose 11·51%, Pepsin 0·3%, Menthol 0·005%, Calc. phosph. 1%, Mag. carb. lev. 0·5%, Sod. Bicarb. *q.s.* ad 100%. *Dose*: $\frac{1}{2}$ teaspoonful.

Vitadatio (*S. A. Palmer Ltd., Leeds*). "Great herbal remedy and blood purifier. Aromatic, stimulant, stomachic and tonic." *Formula*:—Contains the active constituents of Melaleuca Ericifolia 18·00% and Quassia 0·144%. *Dose*: 2 tablespoonfuls.

Vitaepon Brand (*Westcott & Co., London*). "A highly concentrated flesh-former and tonic. Unsurpassed for the treatment of thinness, anæmia, nerve-weakness, etc." *Formula*:—Lecithin 3%, Calc. Hypophosph. 12%, Sod. Hypophosph. 3%, Pot. Hypophosph. 3%, Sol. Albumen 15%, Sugar 10%, Talc. et Kaolin 54%. *Dose*: 2 tablets.

Vocalzone Brand Nasal Capsules (*Distributors: Brooks & Warburton Ltd., London*). "Indicated:—In all affections of the respiratory tract, such as catarrh of the nose and throat, colds, coughs, sore throat, laryngitis, bronchitis, hoarseness, loss of voice, deafness and hay fever." *Formula*:—Methyl-propyl-phenolhexahydride 0·5%, Phenyl Hydrate 0·07%, Ol. Pini 0·04%, Ephedrine 0·5%, Ol. Cinnamon 0·15%, Paraffin Liq. *q.s.* In soft gelatine capsules.

Vogeler's Curative Compound (*International Chemical Co. Ltd., London*). "Relieves dyspepsia, indigestion, biliousness, constipation, sick headache, flatulency, nervous depression, and all stomach, liver and kidney affections." *Formula*:—Each fluid ounce contains: Ginger 3·47 grs., Elder 1·62 grs., Comfrey 0·4 grs., Sarsap 0·4 grs., Valerian 0·12 grs., Capsicum 0·022 grs., Myrrh. 0·12 grs., Aloes 9·25 grs., Alcohol 81·6 mins. *Dose*: $\frac{1}{2}$ to 1 teaspoonful; Children, 10 to 15 drops.

Watson's Family Pills (*Horace Watson, Grimsby*). "They are a most efficacious remedy for all disorders of the digestive organs, and for obstruction of the liver and bowels, which produce indigestion, known by the following symptoms: Acidity, heartburn, dizziness, headache, tic-doloureux, toothache, drowsiness, spasms, an uneasy feeling about the pit of the stomach. . . ." *Formula*:—P. Aloes S. ·270, P. Rhei Radix ·170, P. Zingiber ·108, P. Myrrha ·077, P. Sapo Cast. ·077, P. Capsici ·029, P. Podophylli ·024, Ol. Caryophylli ·024, Ext. Gentian ·086, Ext. Theriaca ·137. *Dose*: 1 pill.

Webber's Laxative Pills (*The manufacturer's name is not given on the container*). *Formula*:—Jalapin gr. $\frac{1}{2}$, Aloin gr. $\frac{1}{2}$, Leptandrin gr. $\frac{1}{16}$, Podophyllin gr. $\frac{1}{2}$, Cambog. gr. $\frac{1}{16}$, Benzyl Succinate gr. $\frac{1}{2}$, Ol. Res. Capsici min. $\frac{1}{16}$. *Dose*: 2 to 4 pills.

Welch's Original Female Pills (*C. & G. Kearsley Ltd., London*). "A medicine known for over a century as a remedy for those disorders to which young women are so frequently subject at puberty, and in assisting nature when the functions from various causes are not fulfilling their recognised part in keeping the periodical regularity of action so absolutely necessary to female health up to a certain period of life." *Formula*:—Ferri Sulph. 52·5, Elecampane 5·32, Curcuma 5·32, Glycyrrh. 5·32, Sulph. Sub. 5·32, Excip. 26·22. *Dose*: 3 pills.

Wex (*E. Griffiths Hughes Ltd., Manchester*). "Sparkling grape saline. An elegant and refreshing drink combining grape juice derivative with saline to produce effervescence. Gently and unfailingly, Wex urges the eliminating organs to perform their normal functions." *Formula*:—A therapeutically-balanced preparation which, added to water, produces a stimulating effervescence and Sodii Tartras from Acid Tartaricum (46·77%) and Sodii Bicarbonas (53·23%). *Dose*: 1 teaspoonful; Children, $\frac{1}{2}$ to $\frac{1}{2}$ teaspoonful.

Whelpton's Vegetable Purifying Pills (*G. Whelpton & Son Ltd., Hemel Hempstead*). "The occasional or regular use of Whelpton's Pills by assisting nature to get rid of superfluous matter, relieves the congested condition of the skin and kidneys, and tends to restore the natural functions of these important organs. For abscesses, boils, ulcers and scorbutic eruptions." *Formula*:—

Each pill contains Aloe 35.29%, Zingib. 29.41%, Calumb. 29.41%, Cambog. 5.88%. *Dose*: 2 pills.

Whelpton's Vegetable Stomach Pills (*G. Whelpton and Son Ltd., Hemel Hempstead*). "For flatulence or wind, weak digestion, acidity, heartburn, sick headache, nausea, rejection of food, vomiting, etc." *Formula*:—Aloe 33.33%, Zingib. 33.33%, Calumb. 33.33%. *Dose*: 2 or 3 pills.

White Lions (*Shadforth Prescription Service Ltd., London*). "Shadforth Stomach Tablets. Prescription 1077. For the cure of acidity, gastric catarrh, gastritis, fermentation, fullness and acid indigestion." *Formula*:—Bismuth Carbonate $\frac{3}{4}$ th grain, Soda Bicarbonate $\frac{2}{3}$ th grain, Magnesia Carbonate 3 grains, Oil of Peppermint (a trace), Oil of Aniseed (a trace). In each tablet. *Dose*: 1 to 3 tablets.

White Tar Ointment (*Tillott's Laboratories, London*). "Antiseptic, healing and soothing. For the cure of eczema, piles, ulcerated legs, boils, pimples and skin irritation or eruption. Quickly relieves and heals burns, scalds, bites, cuts, chaps and sunburn." *Formula*:—Paraff. Moll. 46.7%, Lana Philosophica 10%, Metahydroxybenzol 4%, Hydroxytoluene Com. 0.02%.

[P1] **White's Brand Q.B.C.** (*Heppells Ltd., London*). "Quinine, Belladonna and Camphor Compound. The remedy for colds, influenza, catarrh, hay fever and all allied affections." *Formula*:—Quin. Sulph. 2.66%, Chloroform 2.07%, Tinct. Bellad. P.B. 6.25% *v/v*, Syr. Tolu 17.76%, Tinct. Card. Co. 11.84%, Liq. Ammon. Fort. 1.77%, Spt. Vini Rect. 38.92%, Aq. Dest. 17.76%, Camphor 0.59%, Coloris .38%. *Dose*: 1 teaspoonful.

Williams (Dr.) Pink Pills for Pale People (*The Dr. Williams Medicine Co., London*). "Safe and effective tonic for the blood and nerves. For anæmic conditions, diseases caused by or dependent upon thin, impoverished blood, and for nervous disorders resulting from malnutrition." *Formula*:—Each pill contains: Ferr. Sulph. Exsic. $\frac{1}{2}$ grain, Sod. Carb. $\frac{1}{2}$ grain, P. Aloe (Cape) $\frac{1}{2}$ grain, Mang. Diox. Precip. $\frac{1}{2}$ grain, Zinc Phosphid. $\frac{1}{2}$ grain, Cupr. Sulph. $\frac{1}{2}$ grain.

Winslow's (Mrs.) Soothing Syrup (*Curtis & Perkins, New York; Anglo-American Drug Co. Ltd. (Regd. Users) Successors*). "A stomach and bowel regulator for infants and children. For children teething. A safe remedy for digestive troubles, constipation and diarrhoea, feverishness and restlessness." *Formula*:—Sod. Cit. 2.160%, Ol. Anisi 0.059%, Ext. Rhei 0.040%, Ol. Coriand. 0.012%, Sucrose 61.339%, Ext. Sennæ Liq. 0.236%, Ol. Fœniculi 0.069%, Sod. Bicarb. 0.223%, Ol. Carui 0.040%, Aq. Dest. 35.802%.

[P1] **Wooldridge's Brand Gout and Rheumatic Tincture** (*Wooldridge Medicine Co. Ltd., London*). "Speedily relieves all rheumatic, gout, neuralgia, lumbago and sciatic pains." *Formula*:—Colchicine 0.07% *w/v*, Pot. Acet. 4.00%, Pot. Bicarb. 4.00%, Pot. Iodid. 2.00%, Caramel 0.80%, Proof Spirit 38.00%, Aqua ad 100. *Dose*: $\frac{1}{2}$ to 1 teaspoonful.

Wright's Coal Tar Ointment (*Wright, Layman & Umney Ltd., London*). "Invaluable for ailments of the skin and blemishes of the complexion. The most valuable remedy for chronic eczema, psoriasis, and all skin diseases of a scaly or parasitic nature." *Formula*:—Ext. Quillaie 0.39, Solventes 12, Olea Essent. 0.15, Acid. Oleic 2.25, Cresol B.P. 0.75, Paraff. Dur. 0.9, Paraffin Liq. 3.6, Alcohol Ammon. 0.75, Cera Flav. 2.5, Adeps. Lan. 20, Pix Carbonis B.P.C. 3, Paraff. Moll. Flav. ad 100.

[P1] **Wright's Coal Tar Vaporizing Liquid** (*Wright, Layman & Umney Ltd., London*). "Specially prepared for use in Wright's Coal Tar Inhaler." *Formula*:—Pix Carbonis B.P.C. 0.25, Olea Essentialia 10.0, Naphthalenum 1.5, Cresol ad 100.0.

Wyamin Brand Capsules (*John Wyeth & Brother Ltd., London*). "Containing vitamins A, B, C, D and nicotinic acid. Biologically standardised." *Formula*:—Vitamin A 6000 Int. Units, Vitamin D 900 Int. Units, Vitamin B 100 Int. Units, Vitamin B₂ Complex containing Lactoflavin 50 gamma, Vitamin C 100 Int. Units, Nicotinic Acid 5 mg. *Dose*: 1 to 2 capsules.

Yadil Antiseptic (*Yadil (1935) Ltd., London*). "The internal antiseptic. It disinfects the human system internally and externally, and being highly diffusive it is of great value in obscure infections where other antiseptics fail." *Formula*:—Glycerol 3.75%, Paraformaldehyde 1.25%, Thymol 0.0625%, Oleum Allii Essentiale 0.008%, Aqua Thymolis (Sat. Sol.) 60.00%.

Yadil Antiseptic Ointment (*Yadil (1935) Ltd., London*). "Containing 5% of Yadil Antiseptic. It is ideal for all conditions where a healing and soothing unguent is needed." *Formula*:—Active ingredients: Glycerol 0.20%

Paraformaldehyde 0.06%, Thymol 0.006%, Oleum Allii Essentiale 0.004%, Adeps. Lanæ, Paraffin Mollæ equal parts up to 100.00%.

Yadil Antiseptic Pills (*Yadil* (1935) *Ltd., London*). *Formula*:—Glycerol 11.25%, Paraformaldehyde 3.75%, Thymol 0.36%, Oleum Allii Essentiale 0.024%, Amylum 40.00%, Kaolinum up to 100.00%. *Dose*: Infants, 1 pill; Children, 2 pills; Adults, 3 pills.

Yadil Pastilles (*Yadil* (1935) *Ltd., London*). "Cleanse the throat and mouth of bacillary infection; invaluable to speakers." *Formula*:—Active ingredients: Paraformaldehyde 0.153%, Thymol 0.002%, Ol. Allii Essentiale 0.001%, Glycerol 0.457%, Ol. Limonis 0.167%, Acid. Citric. 1.785%, Pastille Base ad 100.0%.

Yadlets (*Yadil* (1935) *Ltd., London*). "Yadil antiseptic tablets." *Formula*:—Glycerol 15.00%, Paraformaldehyde 5.00%, Thymol 0.50%, Oleum Allii Essentiale 0.032%, Amylum 40.00%, Kaolinum up to 100.00%.

Yeast-Vite Tablets (*Yeast-Vite Laboratories, Watford*). "The lightning pick-me-up. The safe and wonderful treatment of medicinal yeast." *Formula*:—Cerevis. Ferment. B.P.C. 44.34%, Caffein 2.82%, Acetphenetidin 16.52%, Pot. Brom. 4.13%, Sod. Bicarb. B.P. 12.17%, Mag. Carb. Pond. 17.48%, Caryoph. 2.54%. *Dose*: 2 tablets.

Yestamin (*English Grains Co. Ltd., Burton-on-Trent*). "A pure food product containing the valuable vitamin B₁ and B₂."

Yestamin Pure Yeast Tablets (*English Grains Co. Ltd., Burton-on-Trent*). "Extremely rich in vitamin B. Guaranteed free from drugs." *Formula*:—These tablets are pure dried yeast with a fractional amount of innocuous gum for binding. Pure yeast tablets 5.5 gr. *Dose*: 2 or 3 tablets.

Zam-Buk Brand Ointment (*Zam-Buk Mfg. Co., Leeds*). "For cuts, bruises, scratches, burns, scalds, sprains, eczema, ulcers, bad legs, rashes, pimples, piles, chafings, chapped hands, chilblains, cold sores, cold-on-the-chest, insect-bites." *Formula*:—Resin Pini Palust. 14.40%, Paraffin Dur. 21.50%, Paraffin Mollæ 53.40%, Flor Camphoræ 1.85%, Ol. Eucalypt. Globul 7.56%, Ol. Thymi Vulgar 0.93%, Ext. Chlorophylli 0.03%, Ol. Sassaf. Officin 0.33%.

Zam-Buk Brand Suppositories (*Zam-Buk Mfg. Co., Leeds*). "For the direct application to internal hæmorrhoids (or piles)." *Formula*:—Thymol 0.2, Camphor 1.0, Menthol 0.5, Chlorophyll 0.5, Ol. Eucalyptus 1.0, Ol. Theobrom. 82.45, Paraffin. Dur. 9.22, Resina 5.13, per cent.

Zee-Kol Brand Blood Tonic Pills (*The Shavex Zee-Kol Co. Ltd., London*). *Formula*:—P. Aloes Barb. gr. 50, P. Saponis gr. 0.18, P. Zingib. gr. 0.55, Sacch. Alb. gr. 0.27. *Dose*: 3 to 4 pills.

Zee-Kol Brand Ointment (*The Shavex Zee-Kol Co. Ltd., London*). "For ulcers, piles, rheumatism, bad legs, sores, burns, cuts, bruises, eczema, chilblains, and all forms of skin diseases." *Formula*:—Adeps Lanæ Hydrosus 77.00, Acid Boric 5.40, Zinci Oxidi 8.20, Sulphur 2.50, Colophonium 2.50, Ol. Eucalypt Glob. 3.40.

[P1] **Zom Brand Pile Ointment** (*Arthur H. Cox & Co. Ltd., Brighton*). *Formula*:—Ext. Hamam. Liq. 5.60%, Gall. 13.28%, Acid Boric. 2.07%, Opium Pulverat B.P. w/w 1.87%, Adeps Lan. 26.56%, Paraff. Moll. Flav. 49.79%, Ol. Thym. Alb. 0.83%.

Zotal Brand Liniment (*Burgoyne Burbidges & Co. Ltd., London*). "Relieves rheumatism, lumbago, sciatica, muscular pains, stiffness of the joints, neuralgia, colds, sore throat, chilblains, sprains, cramp, neuritis, bronchitis, cough, asthma." *Formula*:—Capsici Fruct. 1.25, Ol. Camph. Essent. ad 100. Colour, Scarlet Red and Oil Brown q.s.

Zox (*Zox Mfg. Co., London*). "Death to pain. A real cure for toothache, neuralgia, headache, sciatica and nerve pains." The composition of this preparation was not disclosed. *Dose*: 1 powder.

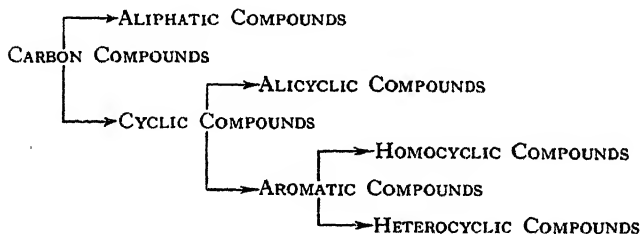
Zubes (*F. W. Hampshire & Co. Ltd., Derby*). "For throat and chest." *Formula*:—Approx. percentages of ingredients: Menthol .3, Marrubium .6, Bals. Tolu .07, Ol. Anisi .19, Gingerin .02, Benzoinum .06, Ol. Menth. Pip .125, Benzyl Benzoas .15, Tinct. Capsici .18, Tussilago .03, Sucrosum et Glucosum Liq. ad 100.

NOMENCLATURE OF ORGANIC COMPOUNDS

The ever increasing number of organic compounds necessitates the adoption of a systematic scheme of nomenclature to obviate the naming of a compound according to the whim of its discoverer. The first serious attempt to provide a universal system was made by the International Congress at Geneva in 1892. Many generalisations found acceptance and such classifications as those of Beilstein and Chemical Abstracts are based upon it. In 1931 (*J. Chem. Soc.*, p. 1607) a Definitive Report of the Committee for the Reform of Nomenclature in Organic Chemistry was issued which aimed at the preservation of common usage as much as possible whilst achieving some simplification. Thus the use of many names which were established by practice was consolidated. A completed system for international adoption has not been evolved and some idea of the difficulties to be overcome may be derived from the knowledge that there are over 3300 different ring systems known at the present time. However, sufficient stability in the principles of naming has been attained by virtue of these efforts that non-ambiguous names which indicate the constitution of the compound can be coined for all organic substances.

The general principle underlying the scheme is the naming of all compounds as derivatives of a comparatively small number of parent structures, such structures being indicated by a termination specific to them. Thus the terminations "*ane*" and "*ol*" indicate a paraffinoid and a hydroxy compound respectively.

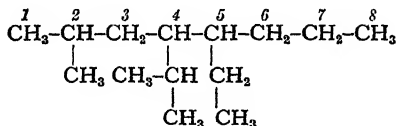
For the purpose of this explanation of the principles of nomenclature, the following classification of organic compounds is used.



ALIPHATIC COMPOUNDS

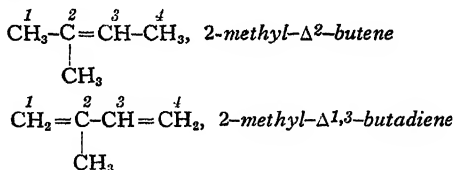
Hydrocarbons. The names of all saturated hydrocarbons (C_nH_{2n+2}) end in *ane*: *methane* (CH_4), *ethane* (C_2H_6), *propane* (C_3H_8), *butane* (C_4H_{10}), higher homologues being named according

to the number of carbon atoms in the compound: *pentane* (C_5H_{12}), *hexane* (C_6H_{14}), *heptane* (C_7H_{16}), etc. Branched chain compounds are regarded as derivatives of a normal, or unbranched chain substance, the position of substitution being indicated by numbering the carbon atoms comprising the normal compound. The names of such compounds must be based upon that of the *longest* unbranched chain present and the numbers chosen so that they are the lowest possible.



The above compound contains a continuous chain of eight carbon atoms and is therefore named as a derivative of octane: *2-methyl-4-isopropyl-5-ethyl-octane*. If the octane chain were numbered from the other end the substituent groups would fall on carbon atoms 4, 5 and 7, which are higher numbers than 2, 4 and 5 and therefore are not used. Should the compound contain several functional groups the longest chain containing the principal functional group or greatest number of double or triple linkages or other substituents is chosen as the basis for the name.

Unsaturated Hydrocarbons. In those containing one double bond the termination *ane* of the parent hydrocarbon is changed to *ene*; similarly those containing two, three, or many double bonds are termed respectively *dienes*, *trienes*, or *polyenes*. The double bond is indicated by the Greek letter Δ and its position in the molecule is fixed by numbering the carbon skeleton of the parent hydrocarbon



Similarly the acetylenic hydrocarbons (triple bond) have the termination *ane* replaced by *yne*: *diyne*, *triyne*, etc., such as, $CH \equiv C-CH_2-CH_2-C \equiv CH$, 1 : 5-hexadiyne.

Throughout this section names of functions and substances used as examples of nomenclature appear in *italic type*, so as to give emphasis and to assist the reader. Normally these names are printed in Roman type and only those parts which indicate the existence of isomeric forms are italicised (*see Use of Italics*, p. 472). Such portions which normally are italicised are shown in Roman type.

Simple and Complex Functions. The method illustrated by the foregoing examples is applicable to a vast array of chemical compounds. Substances of simple function contain an active grouping of one kind only, which may be repeated in the molecule, and their names are derived from the longest unbranched chain containing that function with the termination modified to indicate the constitution of the function. In compounds containing more than one kind of function (complex functions) the termination expresses the principal function, the others being indicated by appropriate prefixes. The groupings $-\text{CHO}$, $-\text{CN}$, and $-\text{COOH}$ are regarded as functions, their carbon atoms not being numbered, but the numbering commences on the carbon carrying that group.

Tables I and II contain the commoner functional groups together with the prefixes and suffixes used.

TABLE I

| Function | Prefix | Suffix |
|--|------------------------------------|--|
| Acid, $-\text{COOH}$ | <i>carboxy</i> | <i>carbonic (carboxylic)</i> |
| $\text{R}-\text{C} \begin{smallmatrix} \text{O} \\ \parallel \\ \text{NH}_2 \end{smallmatrix}$ | | <i>carbonamide</i> |
| $-\text{AsO}(\text{OH})_2$ | <i>arsono</i> | <i>arsonic</i> |
| Alcohol or phenol | <i>hydroxy</i> | <i>ol</i> |
| Aldehyde | <i>aldo, oxo</i> | <i>al</i> |
| Amine | <i>amino</i> | <i>amine</i> |
| Alkoxy (Ether oxide) | <i>methoxy, ethoxy, etc.</i> | |
| Pentavalent N | | <i>onium (open chain)</i> <i>inium (closed chain)</i> |
| Cyanide | <i>cyano</i> | <i>carbonitrile or nitrile</i> |
| Ketone | <i>keto</i> | <i>one</i> |
| $-\text{SOOH}$ | <i>sulphino</i> | <i>sulphinic</i> |
| $-\text{SO}_2\text{OH}$ | <i>sulpho</i> | <i>sulphonic</i> |
| Hydrazine | <i>hydrazino</i> | <i>hydrazine</i> |
| Double bond | | <i>ene</i> |
| Triple bond | | <i>yne</i> |
| Mercaptan | <i>mercapto</i> | <i>thiol</i> |
| Urea | <i>ureido</i> | <i>urea</i> |

The principles upon which the names for many of the above radicals are derived are explained in the following table.

Radicals derived from hydrocarbons, etc.

(a) *from hydrocarbons:*

Univalent—Substitute *yl* for *ane*.
 Substitute *enyl* for *ene*.
 Substitute *ynyl* for *yne*.
 Substitute *dienyl* for *diene* (etc.).

Bivalent (by loss of 2H from same carbon)—
 Substitute *ylidene* for *ane*.

TABLE II

| Function | Prefix | Function | Prefix |
|---|--------------------------------------|-------------------------------------|--------------------------|
| Halogen | <i>chloro, bromo, etc.</i> | $-C_{14}H_9$ | <i>anthryl</i> |
| $-NO$ | <i>nitroso</i> | $-C_{14}H_7$ | <i>phenanthryl</i> |
| $-NO_2$ | <i>nitro</i> | $-C(C_6H_5)_3$.. | <i>triphenylmethyl</i> |
| $-N \begin{smallmatrix} \diagup N \\ \\ \diagdown N \end{smallmatrix}$ | <i>azido, triazo</i> | $-CH_2-CH_2-CH_2-$ | <i>trimethylene</i> |
| $-N=N-$ | <i>azo</i> | $-CH=CH-$ | <i>vinylene</i> |
| $-As=As-$ | <i>arseno</i> | $-C_6H_4-$ | <i>phenylene</i> |
| $-N=N-(OH, Cl,$ etc.) | <i>diazo</i> | $-CH_2-$ | <i>methylene</i> |
| $-N-N-$ | <i>azoxy</i> | $=CH-CH_3$ | <i>ethylidene</i> |
| $\begin{smallmatrix} \\ O \end{smallmatrix}$ | | $=CHC_6H_5$ | <i>benzylidene</i> |
| $-NH_2$ | <i>amino</i> | $-CH_2-CO-CH_3$.. | <i>acetonyl</i> |
| $>NH$ | <i>imino</i> | $-CH_2-CO-C_6H_5$.. | <i>phenacyl</i> |
| $=NOH$ | <i>oximino,</i> <i>isonitroso</i> | $-CHO$ | <i>aldehydo</i> |
| $-NH-OH$ | <i>hydroxylamino</i> | $-COCH_3$ | <i>acetyl, acet-</i> |
| $-NH-NH-$ | <i>hydrazo</i> | $-CO-C_6H_5$ | <i>benzoyl</i> |
| $-NH-N=N-$ | <i>diazoamino</i> | $-CO-C_6H_4-OH$ (1 : 2) | <i>salicyloyl</i> |
| $-NH-N=N-$ (cyclic) | <i>azimino</i> | $-CO-CH=$ CHC_6H_5 | <i>cinnamoyl</i> |
| $-NH-C_6H_5$ | <i>anilino</i> | $-CO-C_{10}H_7$ | <i>naphthoyl</i> |
| $-NH-C_6H_4-CH_3$ | <i>toluidino</i> | $-CO-CO-$ | <i>oxalyl</i> |
| $-NH-CO-NH_2$.. | <i>ureido</i> | $-CO-CH_2-CO-$.. | <i>malonyl</i> |
| $-C(=NH)-NH_2$ | <i>guanyl</i> | $-CO-CH_2-NH_2$.. | <i>glycyl</i> |
| $-NH-C(=NH)-$ NH_2 | <i>guanidino</i> | $-CO-CHNH_2-$ CH_3 | α - <i>alanyl</i> |
| $-S-$ | <i>thio</i> | $-CO-CH_2-NH-$ $CO-C_6H_5$ | <i>hippuryl</i> |
| $-SO-$ | <i>thionyl</i> | $-C_4H_5O$ | <i>furyl</i> |
| $-SCN$ | <i>thiocyano</i> | $-C_4H_5S$ | <i>thienyl</i> |
| $=SO_2$ | <i>sulphonyl</i> | $-CH_2-C_4H_5O$.. | <i>furfuryl</i> |
| $-CH=CH_2$ | <i>vinyl</i> | $-C_4H_5NH$ | <i>pyrryl</i> |
| $-CH=CH-CH_3$.. | <i>propenyl</i> | $-C_5H_4N$ | <i>pyridyl</i> |
| $CH_2-CH=CH_2$ | <i>allyl</i> | $-HgOH$ | <i>hydroxymercuri</i> |
| $-C\equiv CH$ | <i>ethinyl</i> | $-HgCl$ | <i>chloromercuri</i> |
| $-CH_2-C\equiv CH$.. | <i>propargyl</i> | $-C_6H_5$ | <i>phenyl</i> |
| $-C_6H_4-CH(CH_3)_2$ | <i>lumyl</i> | $-C_6H_4-CH_3$ | <i>tolyl</i> |
| $-C_6H_3(CH_3)_3$ (1 : 3 : 5) | <i>mesityl</i> | $-CH_2-C_6H_5$ | <i>benzyl</i> |
| $-CH=CHC_6H_5$.. | <i>styryl</i> | $-CH_2-C_6H_4-OH$ | <i>salicyl</i> |
| $-CH_2-CH=$ CHC_6H_5 | <i>cinnamyl</i> | $-CH_2-C_6H_4-OCH_3$ | <i>anisyl</i> |
| $-C_{10}H_7$ | <i>naphthyl</i> | $-CH_2-C_6H_4-CH_3$ | <i>xyllyl</i> |
| | | O (in ring) | <i>oxa</i> |
| | | N (in ring) | <i>aza</i> |
| | | S (in ring) | <i>thia</i> |

Trivalent (by loss of 3H from the same carbon)—
Substitute *ylidyne* for *ane*.

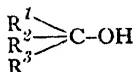
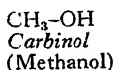
Bivalent (by loss of 1H from each of two terminal carbons)—
Substitute *ylene* for *ane*.

(b) *from acids*:

By loss of OH from COOH—Substitute *yl* for *ic*.

(c) *from alcohols*:

Names universally adopted are retained: *Methanol* and *ethanol* are in use as well as methyl and ethyl alcohol. The amyl alcohols serve to illustrate the systems used. The alternative system is based upon naming all alcohols as derivatives of methyl alcohol which is then called *carbinol*.



This system is sometimes convenient for secondary and tertiary alcohols.

Primary alcohols contain the group $\text{—CH}_2\text{—OH}$

Secondary alcohols contain the group >CH—OH

Tertiary alcohols contain the group >C—OH

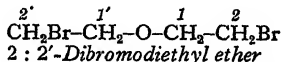
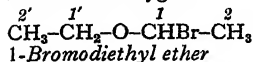
AMYL ALCOHOLS ($\text{C}_5\text{H}_{11}\text{OH}$)

| | Systematic Nomenclature | Alternative Name |
|---|---|--|
| $\text{CH}_3\text{—CH}_2\text{—CH}_2\text{—CH}_2\text{—CH}_2\text{—OH}$ | <i>Pentan-1-ol</i> or <i>Pentanol-1</i> | |
| $\text{CH}_3\text{—CH}_2\text{—CH}_2\text{—CHOH—CH}_3$ | <i>Pentan-2-ol</i> | <i>Methyl-n-propyl-carbinol</i> |
| $\text{CH}_3\text{—CH}_2\text{—CHOH—CH}_2\text{—CH}_3$ | <i>Pentan-3-ol</i> | <i>Diethylcarbinol</i> |
| $\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3\text{—CH—CH}_2\text{—CH}_2\text{OH} \end{array}$ | <i>3-Methylbutan-1-ol</i> | |
| $\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3\text{—CH—CHOH—CH}_3 \end{array}$ | <i>3-Methylbutan-2-ol</i> | <i>Methylisopropyl-carbinol</i> |
| $\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3\text{—C(OH)—CH}_2\text{—CH}_3 \end{array}$ | <i>2-Methylbutan-2-ol</i> | <i>Dimethylethylcarbinol</i> <i>Amylene hydrate</i> |
| $\begin{array}{c} \text{CH}_3\text{—CH—CH}_2\text{—CH}_3 \\ \\ \text{CH}_2\text{OH} \end{array}$ | <i>2-Methylbutan-1-ol</i> | |
| $\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3\text{—C—CH}_2\text{OH} \\ \\ \text{CH}_3 \end{array}$ | <i>2-Dimethylpropan-1-ol</i> | |

If sulphur replaced the oxygen in the above alcohols the names of the compounds would end in *thiol* instead of *ol*. Polyhydric alcohols are named by interposing *di*, *tri*, *tetra*, etc., between the name of the parent hydrocarbon and the suffix *ol*.

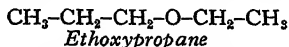
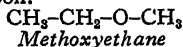
Thus, $\text{CH}_2\text{OH}-\text{CHOH}-\text{CH}_2\text{OH}$ is *Propantriol* (Glycerin) and $\text{CH}_2\text{OH}-\text{CHOH}-\text{CHOH}-\text{CH}_2\text{OH}$ is *Butantetraol* (Erythritol).

Ethers. (a) Simple ethers in which the two radicals carried by the oxygen atom are the same are named as follows:—*Dimethyl ether*, $\text{CH}_3-\text{O}-\text{CH}_3$; *Diethyl ether*, $\text{C}_2\text{H}_5-\text{O}-\text{C}_2\text{H}_5$. When either radical carries a substituent the numbering starts from the carbons next to the oxygen.



When two similar parts of a molecule are numbered they are differentiated by the use of 1 and 1', 2 and 2', etc.

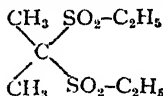
(b) Mixed ethers, in which the two radicals carried by the oxygen atom are different are named as alkoxy derivatives of a hydrocarbon.



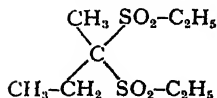
Sulphides, $\text{R}^1-\text{S}-\text{R}^2$, are named in the same manner as ethers, substituting *thio* for *oxy*, or *thioether* for *ether*.

Sulphoxides and **Sulphones** are similarly treated, substituting *sulphinyl* and *sulphonyl* respectively for *oxy*. Thus $\text{CH}_3-\text{CH}_2-\text{CH}_2-\text{SO}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_3$ is (1-*Propylsulphinyl*)-butane and $\text{CH}_3-\text{SO}_2-\text{CH}_2-\text{CH}_3$ is *Methylsulphonylthane*.

On this plan the sulphone hypnotics would receive the names indicated below but the alternative system based upon methane as the parent is equally non-ambiguous.

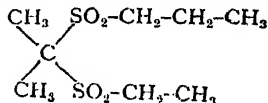


2 : 2-Di(ethylsulphonyl)propane, or
Diethylsulphonyldimethylmethane
(Sulphonal)



2 : 2-Di(ethylsulphonyl)butane, or
Diethylsulphonylmethylethylmethane
(Methylsulphonal)

The coined names are poor as they would suggest an aldehydic constitution: again the term methylsulphonal is ambiguous and could be applied equally well to the following isomeric substance.

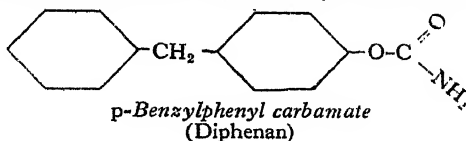


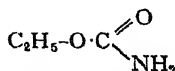
2-Ethylsulphonyl-2-propyl-
sulphonylpropane

Acids. The established names are used, otherwise the following terminations are added to the name of the hydrocarbon which is considered to be substituted.

| Substituent | Suffix |
|--|-----------------------------|
| -COOH | { <i>carboxylic acid</i> |
| -CONH ₂ | <i>carbonic acid</i> |
| -COSH | <i>carbonamide</i> |
| -CSOH | <i>carbothioic</i> |
| -CSSH | <i>carbothionic</i> |
| -C $\begin{smallmatrix} \text{NH}_2 \\ \diagup \\ \text{Cl}_2 \end{smallmatrix}$ | <i>carbodithioic</i> |
| -C $\begin{smallmatrix} \text{NH} \\ \diagup \\ \text{Cl} \end{smallmatrix}$ | <i>carbonamidochloride</i> |
| -C $\begin{smallmatrix} \text{NH} \\ \diagup \\ \text{NH}_2 \end{smallmatrix}$ | <i>carbonimidochloride</i> |
| -C $\begin{smallmatrix} \text{NOH} \\ \diagup \\ \text{OH} \end{smallmatrix}$ | <i>carbonamidine</i> |
| -C $\begin{smallmatrix} \text{NO} \\ \diagup \\ \text{NH}_2 \end{smallmatrix}$ | <i>hydroxamic acid</i> |
| -C $\begin{smallmatrix} \text{O} \\ \diagup \\ \text{N}_2 \end{smallmatrix}$ | <i>carbamidoxime</i> |
| -CH ₃ -C $\begin{smallmatrix} \text{NH}_2 \\ \diagup \\ \text{Cl}_2 \end{smallmatrix}$ | <i>acid azide</i> |
| -CH ₃ -C $\begin{smallmatrix} \text{NH} \\ \diagup \\ \text{Cl} \end{smallmatrix}$ | <i>acetamidochloride</i> |
| -CH ₃ -C(=NH)NH ₂ | <i>acetimidochloride</i> |
| -CH ₃ -C $\begin{smallmatrix} \text{NOH} \\ \diagup \\ \text{OH} \end{smallmatrix}$ | <i>acetamidine</i> |
| -CH ₃ -C $\begin{smallmatrix} \text{NOH} \\ \diagup \\ \text{NH}_2 \end{smallmatrix}$ | <i>acetohydroxamic acid</i> |
| -CH ₃ -CH ₂ -C $\begin{smallmatrix} \text{O} \\ \diagup \\ \text{N}_2 \end{smallmatrix}$ | <i>acetamidoxime</i> |
| | <i>propionazide</i> |

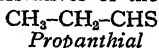
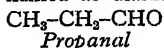
The name carbamic acid is given to amino-formic acid, NH₂·COOH, esters of which are generally classified as urethanes.



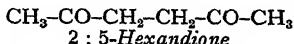


Ethyl carbamate
(Urethane)

Aldehydes are designated by the suffix *al* to the name of the parent hydrocarbon: thioaldehydes by *thial*. The acetals are named as dialkoxy-derivatives of the paraffin.



Ketones receive the termination *one*; diketones, triketones, and thioketones are indicated by the suffixes *dione*, *trione*, and *thione* respectively.

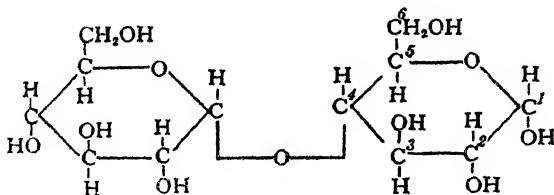


Diketones in which the two functions occupy neighbouring carbon atoms are generically called α -diketones; those in which the two functions are separated by one, two, three, etc., carbon atoms are β , γ , δ , etc., diketones.

Nitrogenous bases are exclusively indicated by the suffix *ine*: aliphatic quinquivalent nitrogen compounds change the *ine* into *onium*: cyclic quinquivalent nitrogen compounds change *ine* into *inium*, or *ole* into *olium*.

Sugars always end in *ose*. The systematic names based upon their cyclic formulæ are derived from the furan and pyran nuclei, the specific name indicating the particular stereoisomeride, glucose thus becomes *glucopyranose*, and fructose, *fructopyranose*.

The names of disaccharides indicate whether the α or β form of the monosaccharide is concerned in the linking: a numeral is used to identify the carbon of the other monosaccharide occupied with the union. The carbon atoms of the monosaccharides are numbered starting from the reducing group.



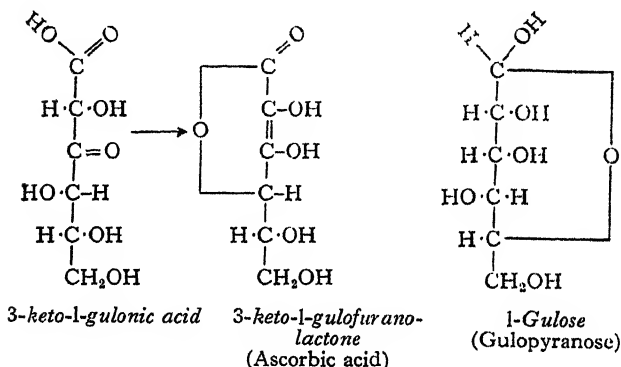
β -Glucose-4- α -glucoside or β -Glucopyranose-4- α -glucopyranoside
(Maltose)

Similarly, lactose is *glucose-4-β-galactoside*, and sucrose is *α-glucopyranose-1-β-fructofuranoside*.

The terms *monosaccharide*, *disaccharide*, *trisaccharide*, *polysaccharide* indicate the number of sugar units in a compound. Individual monosaccharides are called *pentoses*, *hexoses*, etc., according to the number of carbon atoms they contain.

The term *glycoside* is used to denote all ethers of the reducing group, whilst *glucoside*, *mannoside*, *fructoside*, etc., denote the ether of that particular hexose.

Ascorbic acid provides an interesting example of nomenclature related to this group.



In ascorbic acid, "*l-gulo*" signifies the same stereochemical configuration as *l-gulose*; "*furanolactone*" indicate *γ*-lactone structure; the 3-keto group exists in the enolic form.

General Guide to Numbering

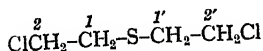
Arabic numerals are used, the lowest numbers being given to the triple bond, the double bond, the principal function or the atoms or radicals which are denoted by prefixes. Positions in side chains are indicated by letters starting from the point of attachment to the nucleus, the letters and the name of the chain being given in parenthesis. The prefixes *di-*, *tri-*, *tetra-*, etc., are used before simple expressions (diethylamine), whilst *bis-*, *tris-*, *tetrakis-*, etc., are used before complex substituents.

$\text{CH}_3\text{-CH}(\text{NHCH}_3)\text{-CH}_2(\text{NHCH}_3)$ $(\text{CH}_3)_2\text{N-CH}_2\text{-CH}_2\text{-N}(\text{CH}_3)_2$
 1:2-bis(Methylamino)propane bis(Dimethylamino)ethane

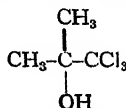
The prefix *bi-* is only used for the doubling of a radical or compound, e.g., *biphenyl*, $\text{C}_6\text{H}_5\text{-C}_6\text{H}_5$.

Complex Functions

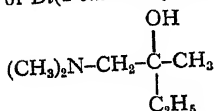
In compounds containing several radicals of different chemical character the principal function is indicated by the termination and the other functions by appropriate prefixes. A few examples will serve as illustrations.



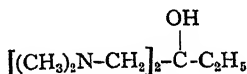
2 : 2'-Dichlorodiethylsulphide
or Di(2-chloroethyl) sulphide



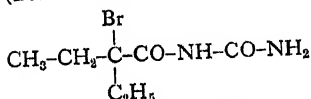
2-Trichloromethylpropan-2-ol
(Chlorbutol)



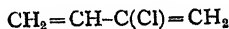
Dimethylaminomethylethyl-
methylcarbinol
(Benzoate is amylocaine base)



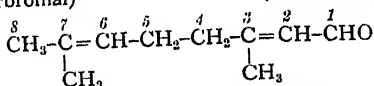
bis(Dimethylaminomethyl)-
ethylcarbinol
(Benzoate is amydricaine base)



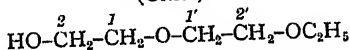
α -Bromo- α -ethylbutyrylurea
(Carbromal)



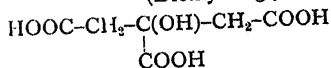
2-Chlorobutadiene
(Chloroprene)



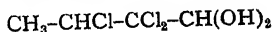
3 : 7-Dimethyl- Δ^2 , 6-octadienal
(Citral)



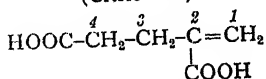
2-Hydroxy-2'-ethoxydiethylether
(Diethyleneglycolmonoethylether)



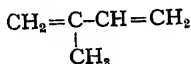
2-Hydroxypropane-1 : 2 : 3-
tricarboxylic acid
(Citric acid)



2 : 2 : 3-Trichlorobutan-
1 : 1-diol
(Butylchloral hydrate)



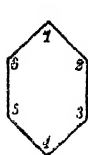
Δ^1 -Butylene-2 : 4-dicarboxylic acid



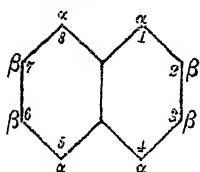
2-Methylbutadiene
(Isoprene)

HOMOCYCLIC AROMATIC COMPOUNDS

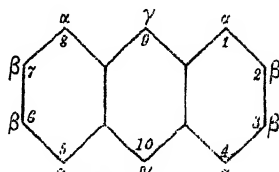
The chief homocyclic systems are as follows:—



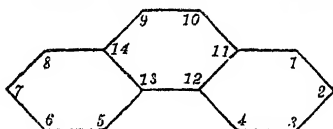
Benzene



Naphthalene



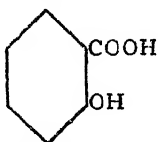
Anthracene



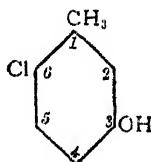
Phenanthrene

The symmetry of the naphthalene and the anthracene formulæ makes possible the use of alternative designations for specific positions. The α -, β -, designations are mainly used for mono-substitution derivatives just as the *ortho* (*o*), *meta* (*m*), and *para* (*p*) prefixes may be used for the di-substitution products of benzene. Other cyclic structures are illustrated below, some of the names being based upon those given above. The nomenclature of naphthalene, anthracene and phenanthrene derivatives and those of other cyclic structures are described under "Condensed Nuclei."

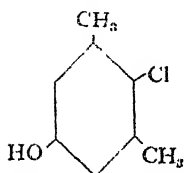
Benzene Derivatives. In polysubstituted substances numbering commences with the principal functional group. *Cresols* are considered as hydroxy-derivatives of toluene, hence the numbering commences with the methyl group. If considered as a derivative of phenol the example below (*6-chloro-m-cresol*) would receive the name *4-chloro-5-methylphenol*. If alternative numbering is possible the lowest numbers are given to the $-\text{OH}$ or $-\text{NH}_2$ group or to the main functional group.



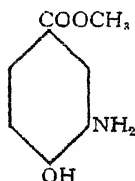
o-Hydroxybenzoic acid
or 2-Hydroxybenzoic acid
(Salicylic acid)



6-Chloro-3-hydroxytoluene
(Chlorocresol)

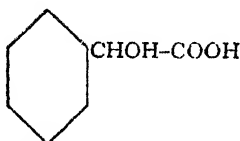


2-Chloro-5-hydroxy-1 : 3-dimethylbenzene
(Chloroxylenol)

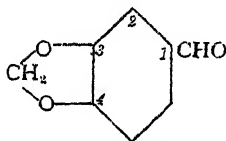


3-Amino-4-hydroxymethylbenzoate
or m-Amino-p-hydroxymethylbenzoate (Orthocaine)

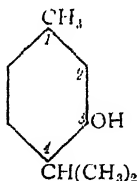
Chlorocresol could be called 6-Chloro-m-cresol or p-Chloro-m-methylphenol, but it is customary to base the name on the hydrocarbon from which the compound is derived, i.e., toluene.



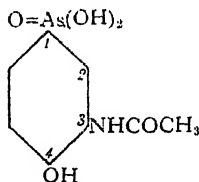
α -Hydroxyphenylacetic acid
(Mandelic acid)



3 : 4-Methylenedioxybenzaldehyde
(Piperonal, Heliotropin)

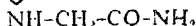
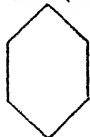
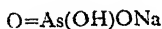


4-isoPropyl-m-cresol
(Thymol)

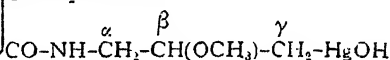
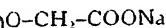


3-Acetyl-amino-4-hydroxyphenylarsonic acid (Acetarsol)

Compounds such as *mersalyl* and *tryparsamide* illustrate another method of fixing the position of substituent groups: "N" and "O" in the systematic names indicate that the phenyl-*p*-arsonate substituent is carried by the nitrogen atom in glycineamide and that the acetic acid group is carried by the oxygen atom of salicylamide.



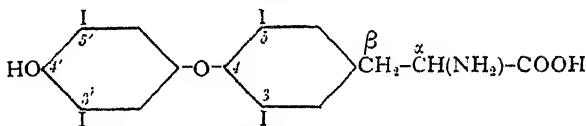
Sodium N-phenylglycineamide-p-arsonate (Tryparsamide)



Sodium salicyl-(γ-hydroxymercuri-β-methoxy) propylamide-O-acetate (Mersalyl)

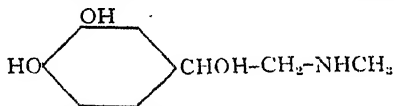
Where aromatic compounds such as *mandelic acid* possess substituent chains of carbon atoms the carbons of the chain are indicated by the letters of the Greek alphabet starting with the point of attachment, whilst numbers are reserved for the cyclic nucleus. In some cases the cyclic compound may be considered as a cyclic derivative of the aliphatic compound; thus *thyroxine* may be considered as a derivative of alanine.

The interpretation of complicated names may be simplified by the judicious use of brackets, as for thyroxine.

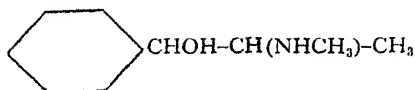


dl-β-[3 : 5-Diiodo-4-(3' : 5'-diiodo-4'-hydroxyphenoxy)phenyl]-α-aminopropionic acid (Thyroxine)

The whole of the grouping within the square brackets is a substituent of the β-carbon atom of α-aminopropionic acid, and the 3' : 5'-diiodo-4'-hydroxyphenoxy group substitutes the hydrogen on the 4-carbon atom in the 3 : 5-diiodophenyl group.



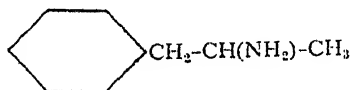
β-Hydroxy-β-(3 : 4-dihydroxyphenyl) ethylmethanamine or *α-(3 : 4-Dihydroxyphenyl)-β-methylaminoethanol* (Adrenaline)



α-Hydroxy-β-methylaminopropylbenzene (Ephedrine)

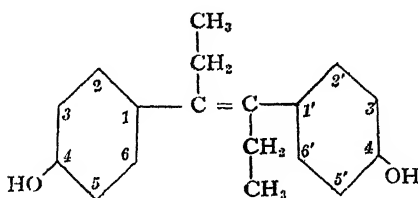
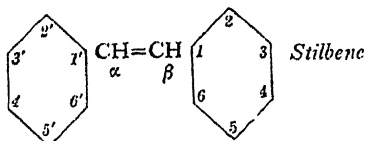


p-Aminobenzenesulphonamide (Sulphanilamide)

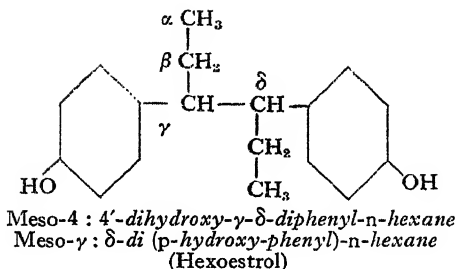


β-Aminopropylbenzene (Amphetamin)

The compound, 1 : 2-diphenylethylene, has been given the name *stilbene*; the two carbons constituting the link are designated α and β .

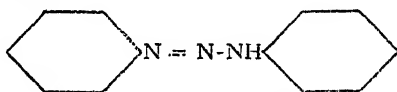


4 : 4'-Dihydroxy- α : β -diethylstilbene
(Stilboestrol)

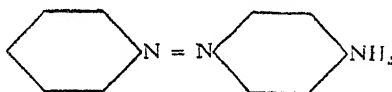


On reduction stilbene becomes hexoestrol when the stilbene structure is destroyed and the compound is named as a derivative of hexane.

The difference between the following two compounds should be carefully noted

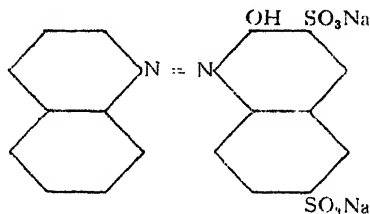


Diazoaminobenzene



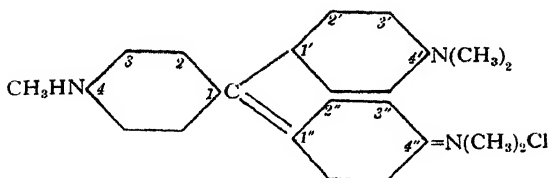
4-Aminoazobenzene

Compounds containing one, two, three, and four *azo* groups are known as *monoazo-*, *bisazo-*, *trisazo-*, and *tetrakisazo-*.



Sodium salt of *α-naphthaleneazo-β-naphthol-3-6-disulphonic acid*

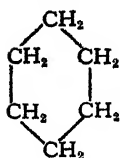
Compounds which contain more than one ring symmetrically placed in arylalkyl union have the numbers of the different rings indicated by one or more dashes.



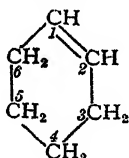
Chloride of 4-methylamino-4' : 4''-di(dimethylamino)triphenyl carbinol anhydride (Component of methyl violet)

ALICYCLIC COMPOUNDS

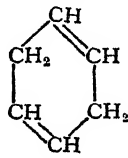
Such compounds, though cyclic in structure, exhibit aliphatic properties and the name recognises this fact.



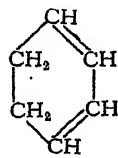
Cyclohexane



Cyclohexene

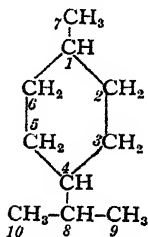


Cyclohexa-
1 : 4-diene

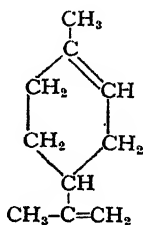


Cyclohexa-
1 : 3-diene

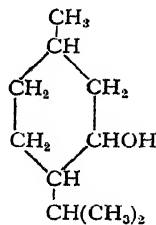
In the case of cyclohexene there is no need to indicate the position of the double bond by numbers, but when this is necessary the lowest possible numbers are given to the ethylene linkages in the unsaturated nucleus.



1-Methyl-4-isopropyl-
cyclohexane
(*p*-Menthane)

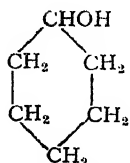


$\Delta^{1,8}$ -*p*-Menthadiene
(Dipentene)

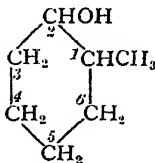


p-Menthane-3-ol
(Menthol)

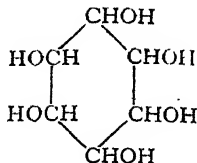
Many cyclic structures which are the parent structures of natural products are given names reminiscent of a naturally occurring derivative. It should be noted that the convention of retaining numbers for the carbons of the ring and Greek letters for the side chains is not observed.



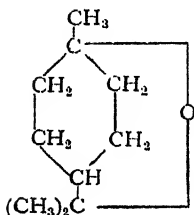
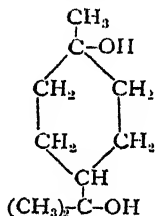
Cyclohexanol



1-Methylcyclohexan-2-ol

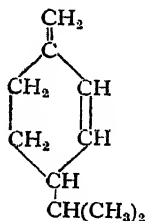
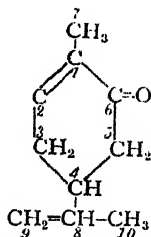


Hexahydroxycyclohexane (Inositol)

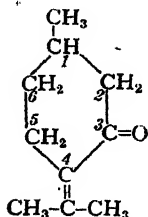
Anhydro-p-menthan-1:8-diol
(Eucalyptol or Cineole)p-Menthan-1:8-diol
(Terpin)

The prefix *anhydro* signifies the loss of the elements of water from the 1:8-diol.

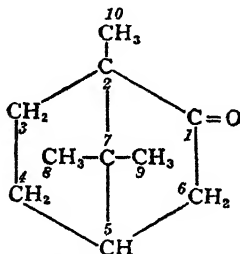
In fixing the position of the double bonds in the molecule a single number is sometimes ambiguous: in such cases a second figure is used.

 $\Delta^1(7),2$ -p-Menthadiene
(β -Phellandrene) $\Delta^1,8$ -p-Menthadiene-6-one
(Carvone)

The figure in brackets indicates that the double bond is situated between carbon atoms 1 and 7, since the position of carbon atom 1 makes alternatives possible.



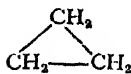
$\Delta^4(8)$ -*p*-Menthen-3-one
(Pulegone)



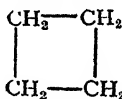
2:5-Endoisopropylidene-2-methylcyclohexanone
(Camphor)

The systematic name for camphor illustrates the method used for dealing with bridged rings. The term *endo* indicates the bridge, the atoms to which it is attached being specified. The numbering of the camphor nucleus is one that has been accepted by custom. It is based upon the structure of cyclohexanone and not upon the terpene skeleton.

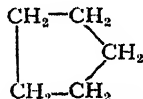
An *alternative method* of nomenclature is in use for both simple and more complex alicyclic compounds. The hydrocarbons are regarded as multiples of CH_2 , *methylene*.



Trimethylene
(Cyclopropane)

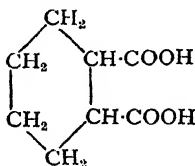


Tetramethylene
(Cyclobutane)

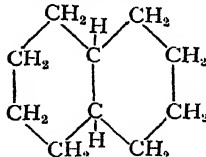


Pentamethylene
(Cyclopentane)

In some cases it is convenient to name alicyclic structures as being derived from the corresponding aromatic nucleus.

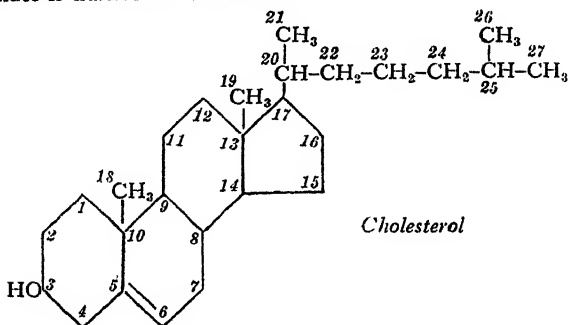


Hexahydrophthalic acid
(Cyclohexan-1:2-dicarboxylic acid)

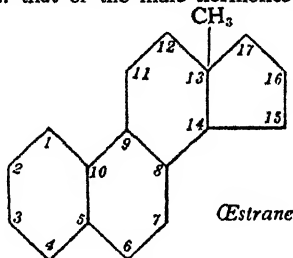


Decalhydronaphthalene
(Decalin)

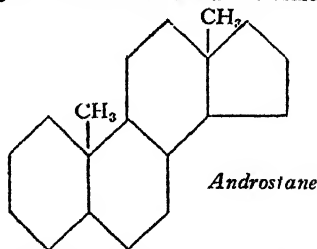
An alicyclic nucleus which is common to the sterols, bile acids, sex hormones, vitamin D and the aglucones of certain cardiac glycosides is numbered as follows:—



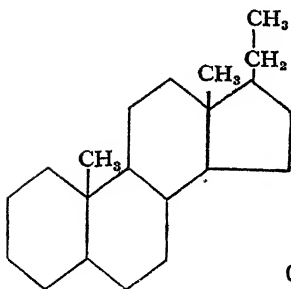
The skeleton from which the female sex hormones are derived is called *œstrane*: that of the male hormones *androstane*.



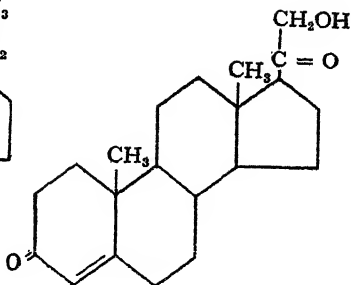
- 3-Hydroxy-17-keto-1 : 3 : 5-*œstratriene* is *œstrone*.
 3 : 16 : 17-Trihydroxy-1 : 3 : 5-*œstratriene* is *œstriol*.
 3 : 17-Dihydroxy-1 : 3 : 5-*œstratriene* is *œstradiol*.



- 3-Hydroxyandrostan-17-one is *Androsterone*.
 17-Hydroxy- Δ^4 -androsten-3-one is *Testosterone*.



Pregnane
or 17-Ethylandrostan-3-one
(Δ^4 -Pregnen-3 : 20-dione is
Progesterone)



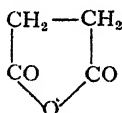
Δ^4 -21-Hydroxypregnen-
3 : 20-dione
21-Hydroxypregesterone
(Desoxycorticosterone or
Deoxycorticosterone)

The use of "Deoxy" or "Desoxy" signifies minus one oxygen atom. Corticosterone possesses a hydroxy group at position 11.

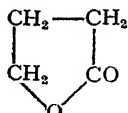
HETEROCYCLIC COMPOUNDS

The common hetero-atoms are O, S and N. Numbering generally commences at the hetero-atom to which the smallest number is given, and proceeds in the direction by which any other hetero-atoms receive the smallest numbers possible.

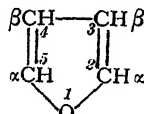
Certain aliphatic compounds possess heterocyclic structures and may be related to the corresponding heterocyclic aromatic nuclei.



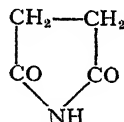
Succinic anhydride
(cf. Furan)



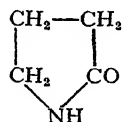
γ -Lactone
(cf. Furan)



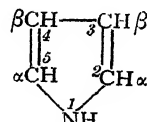
Furan



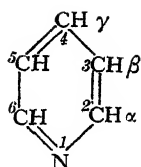
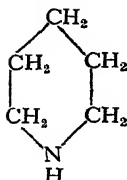
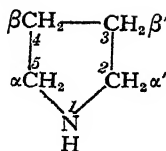
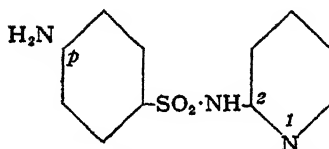
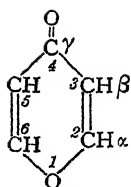
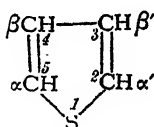
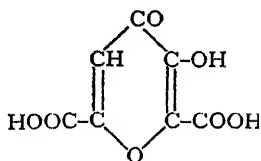
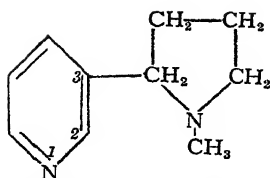
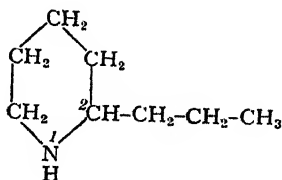
Succinimide
(cf. Pyrrole)

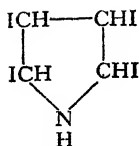


γ -Lactam
(cf. Pyrrole)

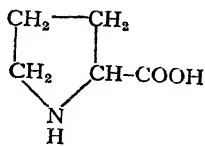


Pyrrole.

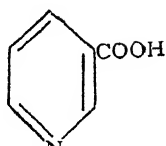
*Pyridine**Piperidine**Pyrrolidine*2-(*p*-aminobenzenesulphonamido)-pyridine
(Sulphapyridine)*γ-Pyrone**Thiophene*3-Hydroxy-*γ*-pyrone-2 : 6.
dicarboxylic acid.
(Meconic acid)1-Methyl-2-(3-pyridyl)pyrrolidine
or α-(3-Pyridyl)-N-methylpyrrolidine.
(Nicotine)2-n-Propylpiperidine
(Coniine)



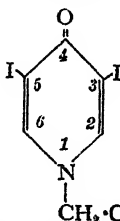
Tetraiodopyrrole



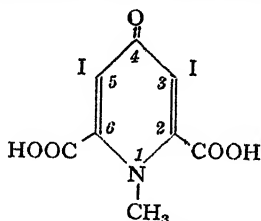
Pyrrolidine-2-carboxylic acid
(Proline)



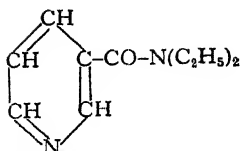
Pyridine-3-carboxylic acid
(Nicotinic acid)



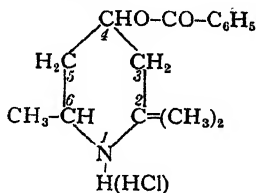
3 : 5-Diiodo-4-pyridone-N-acetic acid
(Component of Liquor Diodoni)



N-Methyl-3 : 5-diiodo-4-pyridone-2 : 6-dicarboxylic acid
(Disodium salt is Iodoxyl)

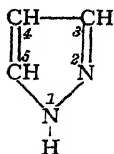
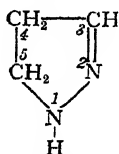
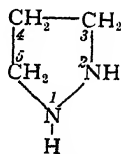
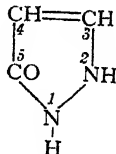


Pyridine-3-diethylcarbonamide
or *Pyridine-β-carboxylic acid*
diethylamide
(Nikethamide)

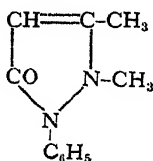


4-Benzoyloxy-2 : 2 : 6-trimethylpiperidine hydrochloride
(Benzamine hydrochloride)

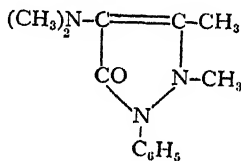
Azoles. Five-membered heterocyclic structures containing more than one nitrogen atom are termed azoles.

*Pyrazole**Pyrazoline**Pyrazolidine**Pyrazolone*

Reduction products are named in the conventional manner—pyrazole, pyrazoline, pyrazolidine. Keto derivatives change the final *ine* into *one*.

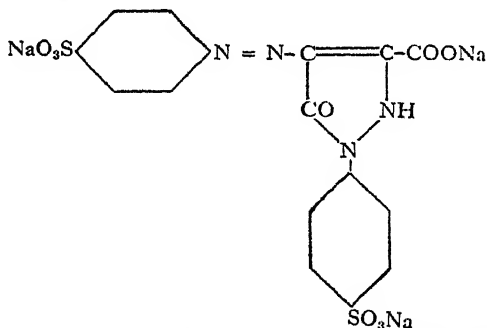


1-Phenyl-2:3-
dimethylpyrazolone-5
(Phenazone)



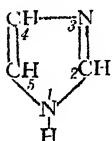
4-Dimethylamino-1-phenyl-
2:3-dimethylpyrazolone-5
(Amidopyrine)

The figure 5 in the above names fixes the position of the carbonyl group in the molecule.

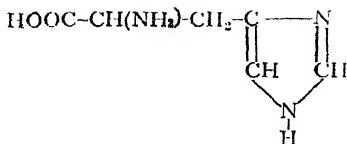
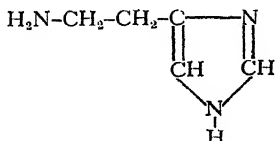


Sodium salt of 4-(p-sulphobenzeneazo)-1-(p-sulphophenyl)-5-pyrazolone-3-carboxylic acid (Tartrazine)

Glyoxalines. Isomeric structures in which the two nitrogen atoms are differently situated are called *glyoxalines* or sometimes *iminoazoles*.

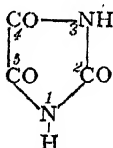
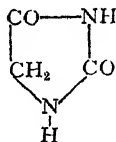
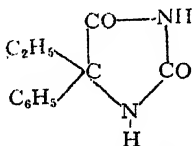
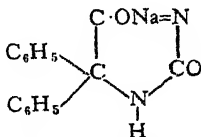


Glyoxaline

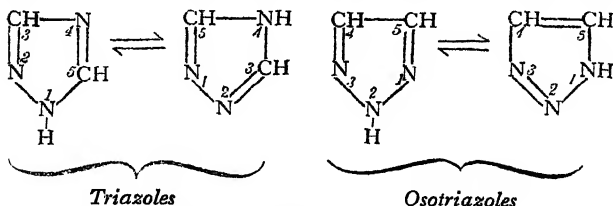
 α -Amino- β -(4-glyoxalyl)propionic acid
(Histidine)4- β -Aminoethylglyoxaline
or β -(4-iminoazoly)ethylamine
(Histamine)

In the above formulæ histidine is treated as a derivative of propionic acid and therefore the α carbon is that to which the carboxylic group is attached. Histamine is treated as an ethylglyoxaline and hence the carbon attached to the ring is designated " α ."

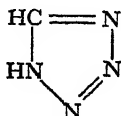
Derivatives of the reduced glyoxaline nucleus are illustrated by the following substances:

Triketotetrahydroglyoxaline
(Oxalylurea or Parabanic acid)2 : 4-Diketotetrahydroglyoxaline
(Hydantoin)5-Phenyl-5-ethyl-2 :
4-diketotetrahydroglyoxaline
or Phenylethylhydantoin
(Nirvanol)Sodium derivative of 5:
5-diphenylhydantoin
(Soluble Phenytoin)

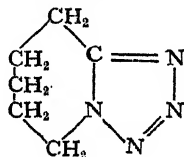
The presence of three nitrogen atoms in a five-membered cyclic structure leads to two tautomeric pairs of compounds. These pairs are distinguished by the names triazoles and osotriazoles.



Tetrazoles contain four nitrogen atoms in the ring.



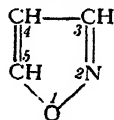
Tetrazole



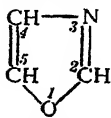
Pentamethylenetetrazole
(Leptazol)

The group pentamethylene, $-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-$, has two free valencies which substitute the two hydrogens in tetrazole.

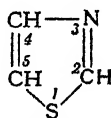
Ring systems in which atoms of oxygen or sulphur replace a nitrogen occur and the numbering always commences with the oxygen (or sulphur) atom. The *isoxazoles* correspond to the pyrazoles, the *oxazoles* to the glyoxalines.



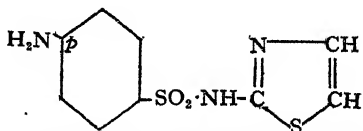
Isoxazole



Oxazole

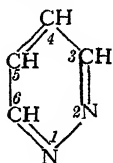


Thiazole

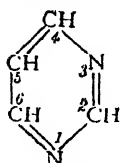


2-(p-aminobenzenesulphonamido)-thiazole
(Sulphathiazole)

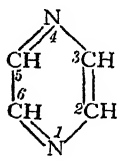
Azines are compounds containing six-membered ring systems containing two or more atoms of nitrogen or nitrogen together with oxygen and sulphur, the latter compounds being termed *oxazines* and *thiazines* respectively.



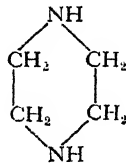
Pyridazine
(Orthodiazine)



Pyrimidine
(Metadiazine)

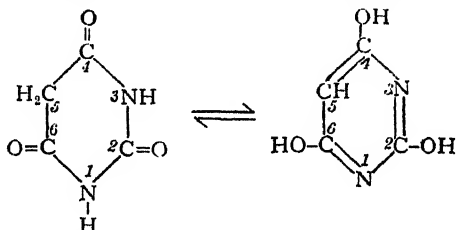


Pyrazine
(Paradiazine)

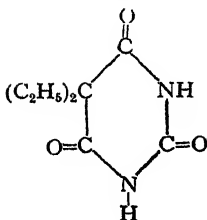


Piperazine

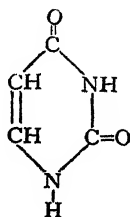
The cyclic *ureides* (barbiturates) contain the pyrimidine skeleton.



2 : 4 : 6-*Trihydroxy-pyrimidine* (Barbituric Acid)



5-*Diethyl-2 : 4 : 6-trihydroxypyrimidine*
(Barbitone)



2 : 6-*Dihydroxy-pyrimidine*
(Uracil)

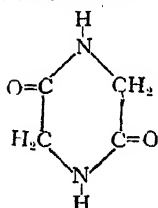
Further examples of derivatives of barbituric acid include the following substances:—

Hexobarbitone = 5- Δ^1 -Cyclohexenyl-5-methyl-N-methyl-barbituric acid.

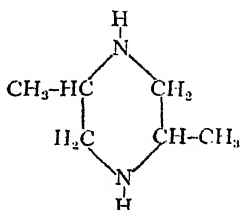
Phenobarbitone = 5-Phenyl-5-ethylbarbituric acid.

Phemitone = 5-Phenyl-5-ethyl-N-methylbarbituric acid.

Piperazines. The cyclic anhydrides formed by the loss of two molecules of water from two molecules of α -amino acids are derivatives of piperazine.

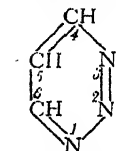


2 : 5-Diketopiperazine
(Glycine anhydride)

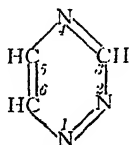


2 : 5-Dimethylpiperazine

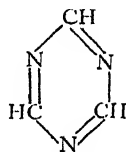
Triazine Nuclei



β -Triazine

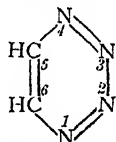


α -Triazine

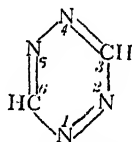


Cyanidines

Tetrazine Nuclei

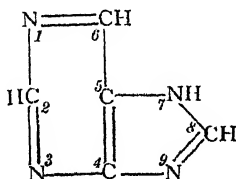


Osotetrazines

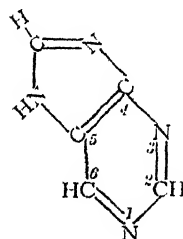


Sym. Tetrazines

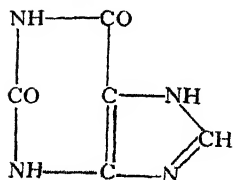
Purines contain the pyrimidine nucleus but are referred to the parent condensed ring system. The alternative formula explains the use of the term *hydroxy*.



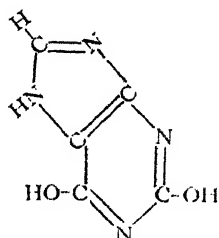
or



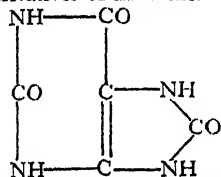
Purine



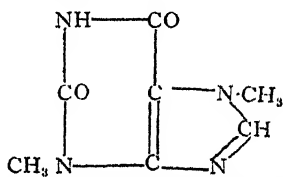
or

**2 : 6-Dioxypurine (Xanthine)**

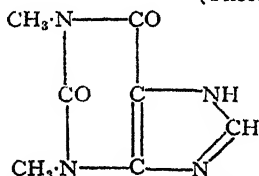
The custom has arisen of naming many other purines as derivatives of xanthine.



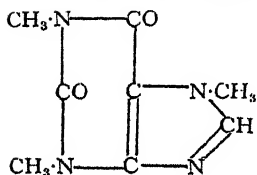
2 : 6 : 8-Trihydroxypurine
(Uric acid)



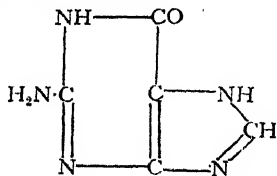
3 : 7-Dimethyl-2 : 6-dihydroxypurine
or **3 : 7-Dimethylxanthine**
(Theobromine)



1 : 3-Dimethyl-2 : 6-dihydroxypurine
or **1 : 3-Dimethylxanthine** (Theophylline)

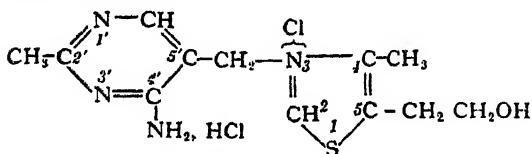


1 : 3 : 7-Trimethyl-2 : 6-dihydroxypurine
or **1 : 3 : 7-Trimethylxanthine**
(Caffeine)



2-Amino-6-hydroxypurine
(Guanine)

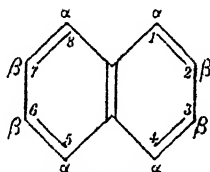
A complex example containing both pyrimidine and thiazole nuclei is afforded by vitamin B₁. The nitrogen of the thiazole is in the form of a quarternary chloride, hence the name "thiazolium chloride."



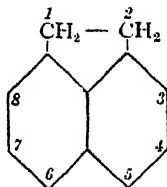
3-(4'-amino-2'-methylpyrimidyl-5'-methyl)-4-methyl-5- β -hydroxyethylthiazolium chloride hydrochloride (Aneurine hydrochloride, Vitamin B₁, Aneurine Chloride hydrochloride)

CONDENSED NUCLEI

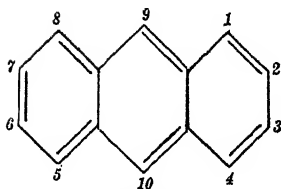
Under this heading compounds are included which contain more than one cyclic system.



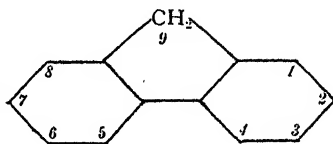
Naphthalene



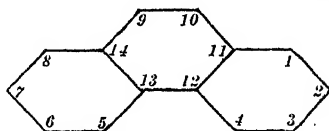
Acenaphthene



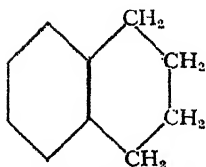
Anthracene



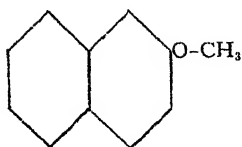
Fluorene



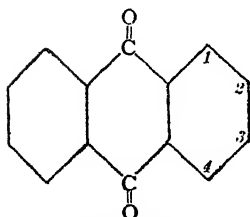
Phenanthrene



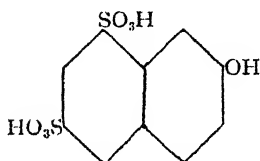
1 : 2 : 3 : 4-Tetrahydronaphthalene
(Tetralin)



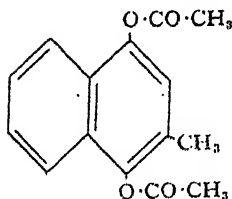
β -Naphthylmethylether
(Nerolin)



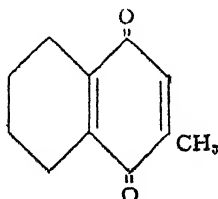
Anthraquinone



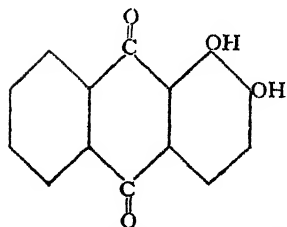
2 : Naphthol-6 : 8-disulphoric acid
(G-Acid)



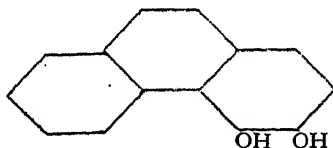
1 : 4-Diacetoxy-2-methyl-
naphthalene
(Acetomenaphthone)



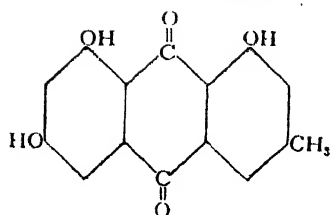
2-Methyl-
1 : 4-naphthaquinone
(Menaphthone)



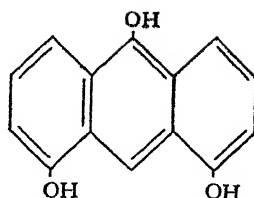
1 : 2-Dihydroxyanthraquinone
(Alizarin)



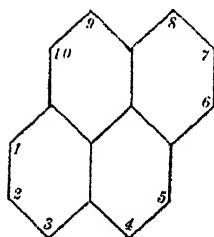
3 : 4-Dihydroxyphenanthrene
(Morphol)



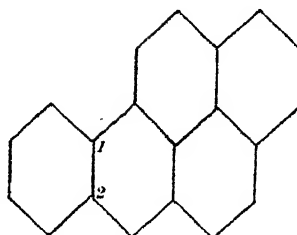
1 : 6 : 8-Trihydroxy-3-methyl-
anthraquinone (Emodin)



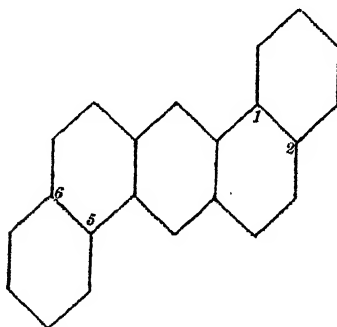
1 : 8-Dihydroxyanthranol
(Dithranol)



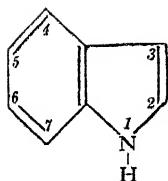
Pyrene



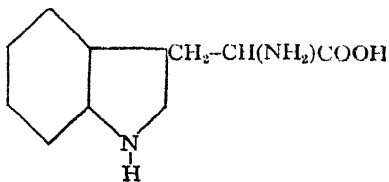
1 : 2-Benzpyrene



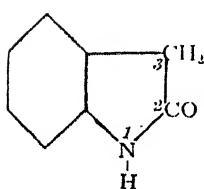
1 : 2 : 5 : 6-Dibenzanthracene
(Carcinogenic hydrocarbons)



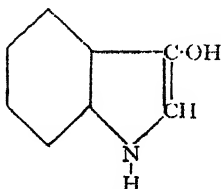
Indole



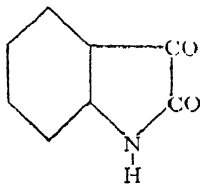
α-Amino-β-[3-indolyl]propionic acid
(Tryptophane)



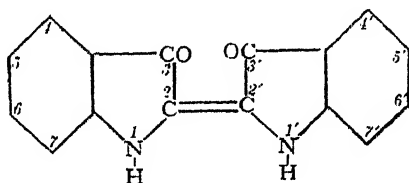
Oxindole



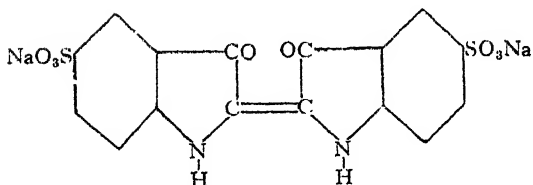
Indoxyl



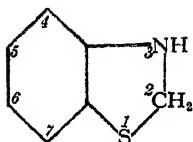
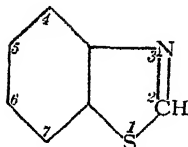
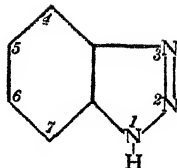
Isatin



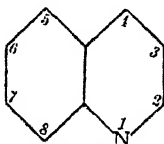
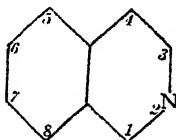
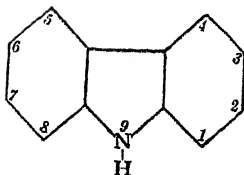
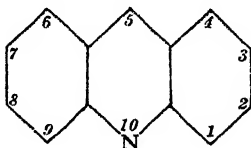
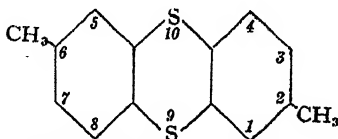
Indigotin

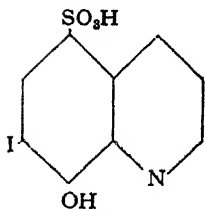


Sodium indigotin-5 : 5'-disulphonate
(Indigo carmine)

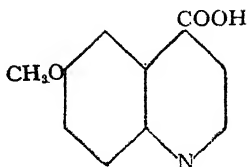
*Benzthiazoline**Benzthiazole**Benztriazole*

In certain of the following ring systems the numbering does not commence with the heterocyclic atom.

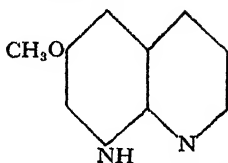
*Quinoline**isoQuinoline**Carbazole**Acridine**2 : 6-Dimethylthianthrene (Mesulphen)*



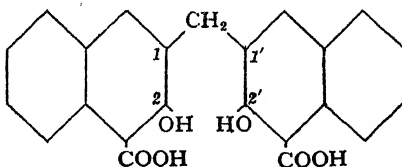
7-Iodo-8-hydroxyquinoline-5-sulphonic acid
(Chiniofon is this substance mixed with NaHCO_3)



6-Methoxyquinoline-4-carboxylic acid (Quininic Acid)

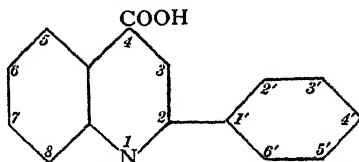


6-Methoxy-8-[(ω -diethylamino- α -methylbutyl)]aminoquinoline
or *N-(ω -diethylamino- α -methylbutyl)-6-methoxyquinoline*
Pamaquin is the salt of this base with the acid illustrated below.

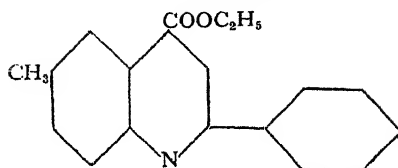


2:2'-Dihydroxy-1:1'-dinaphthylmethane-3:3'-dicarboxylic acid

When a cyclic system is a substituent in a condensed nucleus its positions are indicated by $1'$, $2'$, etc.

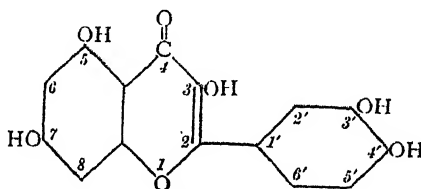


2-Phenylquinoline-4-carboxylic acid (Cinchophen)

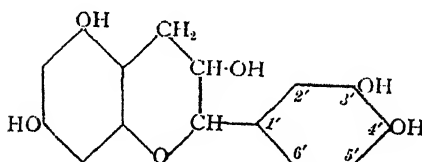


Ethyl 6-methyl-2-phenyl-quinoline-4-carboxylate
(Neocinchophen)

This is also illustrated by quercetin (3 : 5 : 7 : 3' : 4'-Pentahydroxyflavone) and catechin.

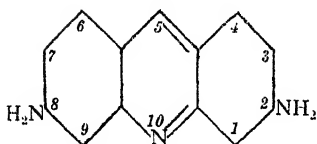


Quercetin

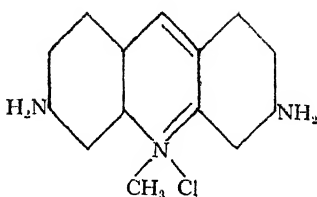


Catechin

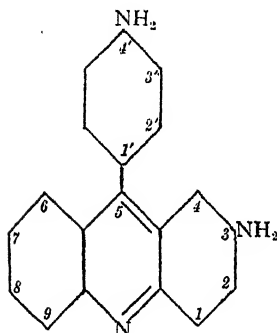
Acridines. Care should be taken in interpreting the names of derivatives of acridine, since no agreement has been reached and different countries use different systems of numbering. All publications referring to acridine derivatives—and indeed derivatives of other cyclic systems—should contain a key plan of the system adopted. The system adopted here is that which is current in Great Britain.



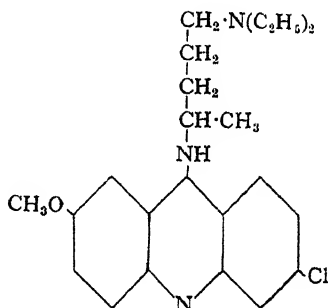
2 : 8-Diaminoacridine
(Proflavine)



2 : 8-Diamino-10-methylacridinium
chloride
(present in Euflavine and Acriflavine)

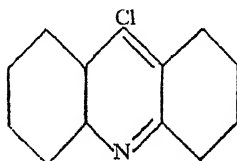


5-(4'-Aminophenyl)-3-
aminoacridine
(Chrysianiline)



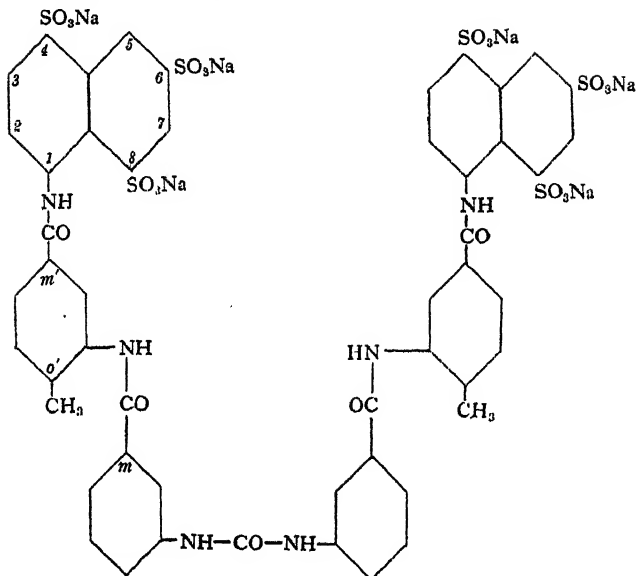
2-Chloro-5-(ω -diethylamino- α -methyl-
butyl)amino-7-methoxyacridine
(Mepacrine)

The "5" position in the acridine nucleus is sometimes designated as "meso," whilst the dyes derived from 5-phenylacridine are technically known as "phosphines."



mesoChloroacridine

Symmetrical Ureas



Symmetrical urea of the sodium salt of m-benzoyl-m-amino-p-methylbenzoyl-1-aminonaphthalene-4 : 6 : 8-trisulphonic acid
(Suramin)

Compounds of this nature are symmetrical derivatives of urea and are known as symmetrical ureas. The official systematic name would be improved if the two *m*s were distinguished as *m'* and *m* respectively to show that they referred to different nuclei. The name is not a good one, but substances of this complexity present serious difficulties in nomenclature. A more accurate description would be: The sodium salt of sym-bis-m-[o'-methyl-m'-(4 : 6 : 8-trisulpho-1-naphthyl-carbonamido) phenyl] carbonamidophenylurea. The words outside the brackets indicate that the substance is a symmetrical di-derivative of diphenylurea substituted in the *m*-position by -CO-NH which carries on the N atom a phenyl substituent itself substituted by an o'-methyl and a m'(4 : 6 : 8-trisulpho-1-naphthylcarbonamido) group.

DERIVATIVES OF ARSENIC, ANTIMONY OR PHOSPHORUS

A meeting of the International Committee on Nomenclature held at Lucerne in 1936 reached agreement with regard to compounds of arsenic, antimony and phosphorus.

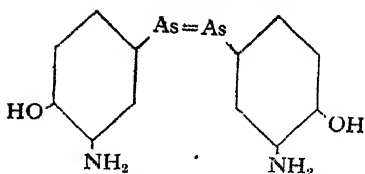
Derivatives of arsine, AsH_3 , are named like amines. Thus— CH_3AsH_2 , *Methylarsine*; $(\text{CH}_3)_2\text{AsCl}$, *Chlorodimethylarsine*; $(\text{CH}_3)_3\text{AsO}$, *Trimethylarsine oxide*; $\text{H}_2\text{As}-\text{CH}_2-\text{CH}_2-\text{AsH}_2$, 1 : 2-*Diarsinoethane*; $(\text{C}_2\text{H}_5)_4\text{AsOH}$, *Tetraethylarsonium hydroxide*.

$\text{RAs}(=\text{O})(\text{OH})$ and $\text{RAs}(=\text{O})(\text{OH})_2$ are named arsenic acids. $\text{RAs}(=\text{O})(\text{OH})_2$ are named arsonic acids. The corresponding prefixes are *arsenico* [$=\text{As}(=\text{O})(\text{OH})$], and *arsono* [$-\text{As}(=\text{O})(\text{OH})_2$]. Thus $(\text{CH}_3)_2\text{AsO}_2\text{H}$ is *Dimethylarsenic acid*; and $\text{C}_6\text{H}_5\text{As}(=\text{O})(\text{OH})_2$ is *Benzenearsonic acid*.

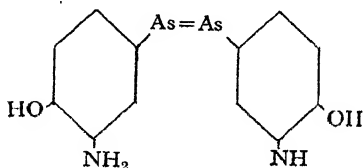
The system is applicable to P and Sb compounds, the syllable “ars” being replaced by “phosph” or “stib” respectively. The following table gives the prefixes and suffixes.

| Radical | Prefix | Suffix |
|---|---------------------------|-------------------|
| $-\text{AsH}_2$ | <i>arsino</i> | <i>arsine</i> |
| $-\text{AsO}$ | <i>arsenoso</i> | |
| $-\text{AsO}_2$ | <i>arso</i> | |
| $=\text{As}(=\text{O})(\text{OH})$.. | <i>arsenico</i> | <i>arsenic</i> |
| $-\text{As}(=\text{O})(\text{OH})_2$.. | <i>arsono</i> | <i>arsonic</i> |
| $-\text{As}=\text{As}-$ | <i>arseno</i> | |
| $-\text{PH}_2$ | <i>phosphino</i> | |
| $-\text{PO}$ | <i>phospho</i> | |
| $=\text{P}(=\text{O})(\text{OH})$.. | <i>phosphinico</i> | <i>phosphinic</i> |
| $-\text{P}(=\text{O})(\text{OH})_2$.. | <i>phosphono</i> | <i>phosphonic</i> |
| $-\text{P}=\text{P}-$ | <i>phosphoso</i> | |
| $-\text{P}=\text{N}-$ | <i>phosphazo</i> | |
| $-\text{P}=\text{As}-$ | <i>phospharseno</i> | |
| $-\text{SbH}_2$ | <i>stibino</i> | <i>stibine</i> |
| $-\text{SbO}$ | <i>stiboso</i> | |
| $-\text{SbO}_2$ | <i>stibo</i> | |
| $=\text{Sb}(=\text{O})(\text{OH})$.. | <i>stibinico</i> | <i>stibinic</i> |
| $-\text{Sb}(=\text{O})(\text{OH})_2$.. | <i>stibono</i> | <i>stibonic</i> |
| $-\text{Sb}=\text{Sb}-$ | <i>antimono</i> | |
| $-\text{Sb}=\text{As}-$ | <i>stibarsino</i> | |

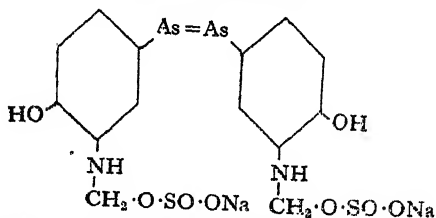
Compounds similarly related to BiH_3 , *bismuthine*, are treated in the same manner.



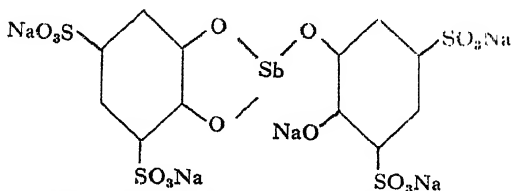
3 : 3'-Diamino-4 : 4'-dihydroxy-arsenobenzene
(The dihydrochloride is Salvarsan)



3 : 3'-Diamino-4 : 4'-dihydroxy-arsenobenzene-N-methylene-
sulphoxylate
(Neoarsphenamine)



3 : 3'-Diamino-4 : 4'-dihydroxy-arsenobenzene-N-N-dimethylene
bisulphite
(Sulpharsphenamine)

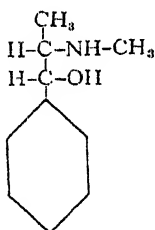


Sodium-antimony-bispyrocatechol-3 : 5-sodium disulphonate
(Stibophen)

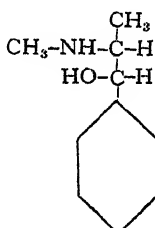
STEREoisomeric Compounds

Optically active compounds are referred to *d*-glucose as the standard. Some confusion exists in the nomenclature since the letters *d* and *l* are used to indicate the relationship of the compound to that standard and have no reference to the direction of rotation. The dextro- or lævo-rotation of the substance is indicated by the symbol + or - : thus *d*(-) is used to indicate that a compound has dextro- configuration but is lævorotatory, and similarly *l*(+) means the exact opposite. At the same time *d* and *l* are used to show the direction of rotation. In order to avoid this confusion it is advisable to use the capital letters D and L for configurational relationship. Thus, lævorotatory *mandelic acid* which has dextro-configuration would then be called D(-) or D(*l*) mandelic acid. The letter *r* indicates the racemic compound of two antimerides, *dl* the optically inactive mixture and *i* the *meso* or internally compensated, non-resolvable form.

Pairs of diastereoisomerides are often differentiated by the term *pseudo* (ψ) as instanced by the following alkaloids of ephedra.

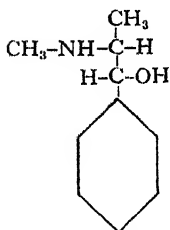


l-Ephedrine

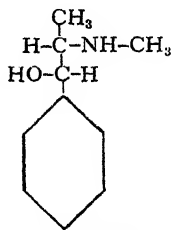


d-Ephedrine

(l- and d- α -hydroxy- β -methylaminopropylbenzene)



l-pseudoEphedrine

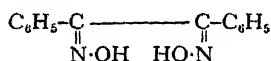


d-pseudoEphedrine

(l- ψ - and d- ψ - α -hydroxy- β -methylaminopropylbenzene)

Geometric isomers of the ethylene type are defined by the terms *cis* and *trans*, whilst for the carbon nitrogen geometric isomerides the terms *syn* and *anti* are used.

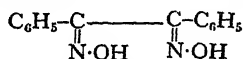
The benzildioximes illustrate the use of a third term, namely *amphi*.



syn-Benzildioxime



anti-Benzildioxime



amphi-Benzildioxime

Allo is also used to signify the less common of the two geometric isomers but the term has historical significance only.

Use of Italics. Italics are used for a prefix which indicates an isomer. Thus, *o*, *m*, *p*, *cis*, *syn*, *anti*, *trans*, *pseudo*, *meso*, etc., should all be italicised, but not the terms "nor" and "homo." "Nor" should be used to indicate the next lower homologue, and "homo," the next higher: the compounds indicated are not isomeric with the parent of the name.

General Conclusions

It will be noticed that alternative names are often possible, but every name used should be, so far as possible, free from ambiguity. The general method of obtaining the name from the formula should be as follows: one of the substituents is chosen as the principal function—carboxyl, if present, is the invariable choice—and is given the lowest possible number; next the longest chain carrying that function and the greatest number of substituents provides the root name. The reverse procedure, deducing the formula from the name, consists of looking along the name for the syllable not preceded by a number to obtain the root and at the termination for the principal function.

From July 1st, 1942, the composition of all "substances recommended as medicines" must be disclosed. A preparation which is official in the *B.P.* or *B.P.C.* may be sold under its official title and the constituents need not be enumerated. The constituents of all other preparations must be disclosed. If the constituents are official in the *B.P.* or *B.P.C.*, an official name, synonym or abbreviation must be used. If such be not the case "the accepted scientific name, or other name descriptive of the true nature of the substance, constituent or ingredient" may be used.

The "accepted scientific name" may be interpreted as being a non-ambiguous, systematic, chemical name: the name should disclose to a competent chemist the chemical structure or identity of the ingredient. This new enactment focuses attention on the

principles of nomenclature. Previously similar provisions applied only to substances mentioned in the Poisons List.

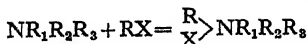
Systematic names are often cumbersome but should always be used where it is desirable to indicate the structure of a compound. "Decalin" and "tetralin" give no indication of the composition of the compounds; memory must be invoked to associate the name with the structure. This in itself is a sufficient plea for systematic nomenclature. The study of organic chemistry would be almost impossible if the student had to learn individual names for some hundreds of thousands of compounds. Systematic nomenclature—international, if possible—is obviously the rational alternative to what, otherwise, would be a state of chaos.

CHEMOTHERAPY

The Relation between Chemical Constitution and Therapeutic Effect

On the continent the term chemotherapy is restricted to the study of substances used in combating an invading organism. It is more reasonable to widen its scope to include the investigation of all substances, the therapeutic effect of which is related to chemical structure. For the purpose of this summary the latter interpretation will be used.

The middle of the nineteenth century was a period of great activity in the determination of the constitution of the active principles of many vegetable drugs. One of the most important characters of such substances is their physiological action and, naturally, attempts were made to relate such activity to the chemical constitution of the molecule. Thus the science of chemotherapy came into being. Probably the first successful generalisation in this field was that of Crum Brown and Fraser (*Proc. roy. Soc. Edinb.*, 1867, 560). They showed that various alkaloids, possessing the most diverse physiological actions, on combination with alkyl halides to form quaternary ammonium derivatives,



where $\text{R}_1\text{R}_2\text{R}_3$ are organic radicles of any complexity and RX stands for alkyl halide, methyl or ethyl iodide, etc., yield substances in almost every case possessing the property of paralysing the motor nerve-endings in the same way as curare. One can obtain, therefore, by methylation of all tertiary bases, quaternary ammonium compounds which are much more poisonous than the original bases. Curare itself contains the tertiary base curine which is not very poisonous, as well as the far more poisonous

ammonium base curarine. Curine on methylation yields curarine which is 226 times as toxic as the original substance. This early success stimulated the hope that it would be possible to correlate physiological action with chemical constitution.

Later, the use of dyestuffs for the selective staining of bacteria suggested the possibility of preparing dyes which might kill or inhibit the growth of pathogenic organisms.

Theories of Physiological Action. One of the earliest general hypotheses was that of Loew (*Natürliche System der Giftwirkung*, Munich, 1893) who held that all substances which react with aldehydes or amines, either by addition or substitution, must possess physiological activity. According to him the greater the reactivity the greater the physiological result, e.g., phenylhydrazine and hydroxylamine are very reactive to ketone and aldehyde groups—hence poisonous both to plants and animals. Aniline is less reactive to aldehydes than phenylhydrazine and is less poisonous than the latter. If the chemical properties of a substance are made more labile by a change in the character of the molecule, then it becomes more toxic, and vice versa, e.g., if the hydrogen of the NH group in many alkaloids be replaced by a methyl group the toxicity is diminished since the substance reacts less readily with aldehydes. Similarly piperidine is more toxic than pyridine, and tetrahydroquinoline is far more toxic than quinoline by reason of the fact that the reduced compounds which contain secondary nitrogen in place of tertiary have a greater reactivity with protoplasm. Compare also pyrogallol (trihydroxybenzene) which is more poisonous than dihydroxybenzene (catechol) and phenol. The toxicity of phenols, is, in the light of this theory, attributed to their reactivity, especially with aldehydes. Salicylic acid (introduction of COOH) is less reactive with aldehydes than phenol, hence less toxic. Loew's theory is restricted to those substances which react with aldehyde and amino-groups and offers no explanation for selective action. As every tissue contains such groups, all drugs should possess a general activity.

Ehrlich (*Proc. roy. Soc.*, 1900, 424; *Studies on Immunity*, 1906, pp. 404-442) suggested a parallel between the action of a drug and the process of dyeing. Witts' theory of dyeing postulates the presence of a chromophore and a salt-forming group in the dye and, by analogy, the drug must contain a "pharmacophore" and an anchoring group. Thus, by changing the type of anchoring group, the seat of action may be moved and the physiological property of the drug altered. In morphine, the anchoring group may be one, or both, of the hydroxyls and the protection of these by the entrance of an organic radicle, methyl, ethyl, or acetyl, causes the hypnotic power to be reduced whilst action on the respiratory centres (produced by morphine to a slight extent) is much increased, e.g., codeine and diamorphine. Again, benzoyllecgonine is twenty times less toxic than its methyl ester (cocaine). It is only necessary for benzoyllecgonine to be esterified—the alcohol used is comparatively unimportant—for the typical action of cocaine to appear, and thus it may be that the anchoring group responsible for the local anæsthetic action does not become operative until the carboxyl is masked.

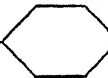

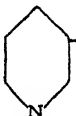

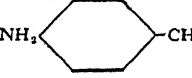
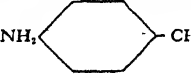
Although these views have not been borne out entirely by subsequent work, they have proved very stimulating to research and have therefore served a useful purpose. One of the main difficulties with such a theory is that it does not explain the varying activity of compounds containing the same groupings differing only in their orientation.

It has been suggested that such difficulties may be met by substituting a theory of indirect action. Thus, bactericides may function by stimulating the formation of antibodies in the host. If this were true the substances should be able to promote immunisation, but as yet no evidence in support has been obtained.

Voegtlin (*Physiol. Rev.*, 1925, 63) has suggested that the activity of the arsenicals is due to their reduction to arsine oxide derivatives which then react with the reduced glutathione present in the tissues. But again the selective action of isomers is not explained.

Recently a theory of indirect action has come into prominence which appears to be based, in certain cases, upon experimental results. According to this theory a drug may exert its activity by interference with an essential enzyme or with an essential metabolite. Thus Loew and Navrátil attribute the action of

physostigmine on the heart to inhibition of choline esterase. The Stedmans found that certain simple urethanes which inhibited many of the properties of physostigmine also showed this inhibitory power. Gaddum (*Pharm. J.*, 1938, 271) suggests that some of the effects of ephedrine are due to its inhibition of an amine oxidase which destroys adrenaline and other amines by removing nitrogen. Woods (*Brit. J. exp. Path.*, 1940, 21, 74) found that *p*-aminobenzoic acid antagonised the bacteriostatic action of sulphanilamide *in vitro*, and Selbie (*ibid.*, 1940, 21, 90) obtained the same result *in vivo*. Fildes (*Lancet*, i/1940, 956) suggested that the activity of sulphanilamide is due to its competition for *p*-aminobenzoic acid which he regarded as a component of an enzyme which was an essential metabolite for those organisms affected by sulphanilamide. The antagonism of *p*-aminobenzoic acid and sulphanilamide has been confirmed by several other workers. McIlwain (*Brit. J. exp. Path.*, 1940, 21, 136; 1941, 22, 148) has found that nicotinic acid and pyridine- β -sulphonic acid are mutually antagonistic and this lends support to the general theory. Barnett and Robinson (*Biochem. J.*, 1942, 36, 364) have shown that pantoyletaurine inhibits pantothenic acid, a constituent of the vitamin B₅ complex which is known to be an essential metabolite for the growth of a number of organisms. The relationship of these growth factors and the inhibitor is shown in the following table

| Growth Stimulator | Corresponding Inhibitor |
|---|---|
| 1.  <i>p</i> -Aminobenzoic acid |  <i>p</i> -Aminobenzenesulphonic acid |
| 2.  Nicotinic acid |  Pyridine- β -sulphonic acid |
| 3.  <i>p</i> -Aminophenylacetic acid |  <i>p</i> -Aminophenylmethanesulphonic acid |
| 4. $\text{CH}_2\text{OH}\cdot\text{C}(\text{CH}_3)_2\cdot\text{CHOH}\cdot\text{CO}\cdot\text{NH}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{COOH}$ Pantothenic acid | $\text{CH}_2\text{OH}\cdot\text{C}(\text{CH}_3)_2\cdot\text{CHOH}\cdot\text{CO}\cdot\text{NH}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{SO}_3\text{OH}$ Pantoyltaurine |

If this theory is found to be universally applicable another avenue of approach to chemotherapeutic problems is available. An investigation concerning the essential factors for growth of an organism might lead to the production of an inhibitor for one or more of them.

Effect of Stereoisomerism. Fischer's famous "lock and key" simile has been invoked to explain the well-known differences in compounds related stereochemically. Sometimes the character, as well as the potency, of the reaction is different, e.g. *l*-hyoscyamine and *d*-hyoscyamine. Difference in potency is best illustrated by *l*-adrenaline which is many times more active than

its enantiomorph. Again *cis* and *trans* dichlorethylenes show considerable differences in physiological behaviour. The characteristic action of the quaternary ammonium compounds does not appear to be dependent upon the presence of nitrogen as it is exhibited by the S, P, and As analogues. Such substances have one common characteristic—the tri-dimensional form of their molecules.

Physical Factors. Many chemically inert substances possess hypnotic properties. There appear to be few chemical relationships between paraldehyde, chloral hydrate, sulphonal, urethanes, and ureides, and hence Overton and Meyer independently suggested that similar physical properties were responsible for the physiological action of bodies belonging to such different common chemical types. They proved that it was possible to obtain approximately the relative potencies of hypnotics by studying the partition coefficients between olive oil and water. Meyer compared partition coefficients with the fraction of the molecular weight required to produce immobility, and obtained the following results:—

| Substance | Partition Coefficient (olive oil : water) | Molecular amount to produce immobility |
|--------------------|--|---|
| Methylsulphonal .. | 4.4 | 0.0013 |
| Tetronal | 4.0 | 0.0018 |
| Sulphonal | 1.1 | 0.006 |
| Bromal hydrate .. | 0.7 | 0.006 |
| Chloral hydrate .. | 0.2 | 0.002 |
| Ethyl urethane .. | 0.14 | 0.04 |
| Alcohol | 0.03 | 0.5 |

It will be seen that the two methods classify the substances in approximately the same order.

Such a theory accords with the fact that the nerves are surrounded by lipid matter which would protect the nerve fibres from substances insoluble in lipoids. On the other hand, the theory does not explain the action of the drug on the nerve fibre itself or the specificity of the action of certain identities.

The physical properties of a drug and its chemical stability may determine whether the substance can reach the required seat of action without suffering decomposition or general absorption, but it is unlikely that such properties offer a complete explanation for the physiological activity.

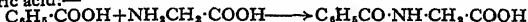
Metabolic Reactions. The ultimate fate of the drug in the body is bound to have an effect upon its activity. In general, the changes which occur lead to the production of a less toxic compound by way of hydrolysis, oxidation, or reduction, sometimes followed by the combination of the product with sulphuric, glycuronic, or aminoacetic acid. Hydrolysis takes place in the alimentary tract. The saliva usually has little action; salts of organic acids are generally decomposed into the free acid and a chloride of the base, but esters and similar bodies are in the majority of cases undecomposed by the gastric contents. In the small intestine, however, the drug encounters the pancreatic enzyme, trypsin, and an alkaline medium. Trypsin has marked hydrolysing action on esters, anilides, and similar bodies,—here, after saponification, the components of the drug exert their specific action. Oxidation and reduction occur in the tissues and in the blood. Aliphatic substances are often completely oxidised to carbon dioxide, water, and urea although the methyl group appears to offer considerable resistance. For instance, acetone is oxidised with difficulty, diethylketone easily, whilst methylethylketone occupies an intermediate position. Primary and secondary alcohols are easily decomposed, whilst tertiary alcohols and chloro-derivatives are comparatively resistant.

Aromatic substances are more stable and the nucleus usually remains intact: side chains are converted into carboxyl groups (toluene gives benzoic acid) whilst other substances are transformed into the *para*-hydroxyl derivative. Aniline yields *p*-aminophenol, and this fact accounts for the introduction of derivatives of this compound into medicine, phenacetin being a well-established example. An interesting action of demethylation occurs in the case of xanthine, theobromine and caffeine, the first being without action on the heart muscle, the second acting slightly and the third showing more marked toxic action.

It was found that the products of metabolism after giving caffeine and theobromine contain xanthine bases poorer in methyl groups than the substances given. In man, caffeine is reduced to theophylline,—this shows that there is a splitting off of methyl groups, which groups appear to be responsible for action on the heart, i.e., there is a relationship between physiological action and the changes undergone by the substance in the organism. Reduction occurs much more rarely—quinone forms hydroquinone and certain substituted nitro-compounds are reduced to the corresponding amino-compounds although nitrobenzene is not converted into aniline.

It has been mentioned that products of these reactions are often eliminated in combination with certain acids.

Phenols form the potassium sulphuric esters unless the phenol is relatively non-toxic when it is eliminated unchanged. Methyl salicylate combines with sulphuric acid, but salicylic acid, owing to the presence of the carboxyl group, forms the ester with aminoacetic acid. Aliphatic compounds eliminated with glycuronic acid, $\text{CHO}(\text{CHOH})\cdot\text{COOH}$, probably react with glucose to form a glucoside which is then oxidised to the glycuronic acid derivative. Aromatic compounds form an additive compound with the aldehyde group of this acid. The condensation with aminoacetic acid can be illustrated by the formation of hippuric acid:—



Thus, benzoic acid, derivatives of benzoic acid, and similar substances formed by oxidation are eliminated as hippuric acid and its derivatives.

The possibilities of such reactions make it necessary to know whether the physiological effect produced by a compound is in fact due to that compound or to an artefact formed after absorption. It was once thought that the action of chloral hydrate was due to its decomposition into chloroform in the body, but it was later determined that the hypnotic action was due to the corresponding alcohol produced by the reduction of chloral. An interesting sequel was the introduction into medicine as a basal narcotic of a solution of tribromoethyl alcohol in amylene hydrate (Bromethol).

From the above survey it is apparent that no one theory explains satisfactorily physiological action, and few generalisations can be made. In fact the original finding of Crum Brown concerning quaternary ammonium compounds still remains almost unique. Other fairly well-established generalisations may include: (a) benzoylation of amino-alcohols always gives rise to local anaesthetics; sometimes the use of the *p*-aminobenzoic derivative is preferable as in procaine; (b) the β -position in arylaliphatic amines (e.g., β -phenylethylamine derivatives) appears to be of importance in sympathomimetic compounds; (c) the ethyl group is important in certain classes of hypnotics; (d) the introduction of strongly acidic groups such as the carboxyl and sulphonic acid groups, often reduces toxicity and activity. Acylation of amines produces the same effect.

In addition to these few generalisations, a vast amount of information has been obtained, but the findings are applicable only to the series concerned. However, the following summary of the effects of common groupings may serve to illustrate the type of knowledge available.

Inorganic Substances. Blake, in 1839, stated that any action was due to the electro-positive radicle, acid groupings being inert. In 1881 it was stated that activity increased with increase of atomic weight amongst isomorphous substances—e.g., Li, Na, Rb, Cs, Ag and Tl; Mg, Mn, Co, Ni, Cu, Zn, Cd; Ca, Sr, Ba. Potassium and ammonium provide exceptions but these are also exceptions to Mitscherlich's law of isomorphous substances possessing similar spectra.

Amongst the electro-negative elements there appears to be no relation between activity and atomic weight. The effect appears to be due to the ions and hence ionisation plays an important part: mercuric cyanide is soluble but little ionised and is much less poisonous than mercuric chloride. Phosphonium, arsonium and stibonium bases exhibit no reactions of P, As, or Sb but resemble the corresponding nitrogen compounds.

Organic Substances. Aliphatic compounds mainly produce hypnotics, and aromatic compounds antipyretics.

Hydrocarbons. Aliphatic hydrocarbons which exhibit volatility and solubility are narcotic, the activity reaching a maximum at C_8 and C_7 . Activity is increased on the introduction of a

double bond. Amongst aromatic hydrocarbons benzene compounds show a paralysing action on the motor nerves and a further effect on the brain and spinal cord. Naphthalene is less toxic than benzene.

Alkyl Groups. In homologous series of compounds produced by varying alkyl radicles, peaks of activity occur—not always at the same point. The ethyl group appears to be particularly effective in hypnotics as illustrated by the sulphones. More generally the peak occurs with the butyl or amyl member.

In derivatives of barbituric acid the two substituent groups on the 5-carbon atom should together contain not less than 4 or more than 8 carbon atoms, and at least one of the groups should be aliphatic. Outside these limits derivatives are either too toxic or too inactive for use.

Replacement of hydrogen of the nucleus by methyl produces an increase in the effects, *cf.* also the methylation of xanthine (*antea*). Replacement of the hydrogen of a hydroxyl group often reduces activity, *cf.*, catechol, $C_6H_4(OH)_2(1:2)$, guaiacol, $C_6H_4OH \cdot OCH_3$, and veratrole, $C_6H_4(OCH_3)_2$. Again *ortho*-methoxybenzoic acid, $C_6H_4OCH_3 \cdot COOH$, and anisic acid, $C_6H_4OCH_3 \cdot COOH(1:4)$, are less active than salicylic acid, $C_6H_4OH \cdot COOH$, but this is not invariably true—resorcin, $C_6H_4(OH)_2(1:3)$, is far less poisonous than dimethyl-resorcin, $C_6H_4(OCH_3)_2(1:3)$.

Alkylation of amines reduces toxicity. Some interesting cases of specificity occur: for instance, certain dyes containing the diethylamino group stain nerve fibres whereas the corresponding methyl derivatives are inactive (Ehrlich and Michaelis); *p*-phenetolcarbamide, $C_2H_5O \cdot C_6H_4 \cdot NH \cdot CO \cdot NH_2$ (dulcin), is intensely sweet whilst the methyl analogue is tasteless. No general rule can be postulated concerning the introduction of the phenyl group.

Hydroxyl Groups. Depend upon the function which they perform.

Alcohols. Narcotic action reaches a maximum at C_7 , and activity increases with the branching of the chain: secondary are more active than primary, tertiary than secondary; e.g., amylene hydrate. Introduction of further hydroxyls reduces activity, the effect being roughly proportional to the number present. Solubility in water increases as does the property of sweetness, *viz.*, monohydric alcohols, glycols, glycerol, mannitol, etc.

Phenols. Introduction of $-OH$ into the aromatic nucleus increases activity and often promotes antiseptic qualities. Amongst the homologues of phenol, increase in molecular weight is accompanied by increased activity and reduced toxicity. Polyhydroxyphenols are still more active and the meta compounds are least active: phloroglucinol is the least active of all the di- and tri-hydroxybenzenes. Alkyl resorcinols are stronger bactericides than resorcinol, the peak being reached with 4-hexylresorcinol.

Halogens. In aliphatic bodies there is increase in narcotic power, but there is also an increase in depressant action on the heart and blood vessels. The narcotic power and toxicity of chlorine compounds is well seen in the case of the chlorhydrins,—narcotics and vasodilators derived from glycerin which is inert, tri-chlorhydrin being most active and the mono-compound least. Note that in the case of the tri- and mono-chloracetic acids the toxicity is reversed. Halogen introduced in the benzene nucleus causes little alteration in properties. Organic iodine compounds differ from those of chlorine and bromine in having greater antiseptic and toxic properties and diminished hypnotic effects, *cf.*, CHCl_3 , CHBr_3 , CHI_3 .

Although the entrance of halogens increases the narcotic action of a drug, the molecule acts as a whole, neither chlorine nor bromine being set free in the tissues. Examples: chloral hydrate, chlorbutol.—J. Grier, *Brit. colon. Drugg.*, i/1913, 282.

Aldehydes. Formaldehyde is very reactive chemically and physiologically. It is a strong irritant to the mucous membrane and coagulates proteins. Acetaldehyde produces excitation and then anæsthesia. Paraldehyde is stronger in action than the latter. By entry of OH into the aldehyde molecule and by condensation of these bodies to form aldols, reactivity is lowered, as also physiological power—the sugars are practically inert. The aromatic aldehydes are of low toxicity.

Ketones. Similar to alcohols—narcotic. Hypnotic action is seen in the mixed ketones, e.g., acetophenone, $\text{C}_6\text{H}_5\cdot\text{CO}\cdot\text{CH}_3$.

Acid Groups. The introduction of such groupings usually increases considerably the solubility and therefore the “dispersibility” of a compound. Hence they cause generally a decrease in activity or total suppression, e.g., substances containing an OH group, on combining with sulphuric acid, lose their toxicity—phenol is toxic but phenylsulphuric acid is harmless, *cf.* also morphine, $\text{C}_{17}\text{H}_{17}\text{NO}(\text{OH})_2$, and morphine-sulphuric acid, $\text{C}_{17}\text{H}_{17}\text{NO}(\text{OH})\cdot\text{O}\cdot\text{SO}_2\cdot\text{OH}$ —this latter is practically inert. The sulphonic acids of various drugs are in nearly every case of little use; the introduction of carboxyl (COOH) is almost analogous. For example, COOH reduces the toxicity of benzene, which can be taken in doses of 8 g. per day in comparison with a dose of 12 g. to 16 g. of benzoic acid. Methylamine, NH_2CH_3 , is toxic; glycine, $\text{NH}_2\text{CH}_2\cdot\text{COOH}$, is harmless. If the carbomethoxy group in cocaine be hydrolysed there is a total loss of activity which is regained on esterification.

Acylation of amino groups—i.e. the introduction of an acid residue—has a similar effect on toxicity. The base is liberated slowly by hydrolysis and thus the action is retarded and the concentration of the base remains below the toxic limit. The acetyl group is most generally used but the lactyl, benzoyl and salicyl are not uncommon. Practically all synthetic antipyretic and analgesic drugs contain the acetyl radicle. Not only so but it occurs in such naturally occurring pain-relieving drug-principles as aconitine and colchicine.

Nitro and Nitroso Groups. Replacement of the hydrogen of the hydroxyl-group by the nitro- and nitroso- groups yields the nitrates and nitrites respectively. Both these classes of substances are vasodilators. Peak of activity is obtained at C_5 (amyl nitrite), the nitrites of secondary and tertiary alcohols being stronger than those of primary. Glyceryl trinitrate and erythritol tetranitrate are examples of nitrates used for the same purpose.

Introduction of the nitro group into aromatic compounds invariably increases toxicity and is not accompanied by any action of dilatation.

The CN Radicle. Isocyanides (isonitriles) cause paralysis of the respiratory centre and the cyanides (nitriles) produce coma. Neither, however, are as poisonous as HCN. The lower members in the fatty series, CH_3CN and C_2H_5CN , are less poisonous than the higher—cyanacetic acid, $CNCH_2\cdot COOH$, is practically non-toxic. Cyanogen chloride, $CNCl$, on the other hand, is very toxic as it readily yields HCN.

Basic Nitrogen Groups. These can produce in either series important changes. The introduction of alkyl groupings into such bodies increases basicity and reduces toxicity and as before gives hypnotic effect, e.g., carbamic acid, NH_2COOH (poisonous), gives urethane (ethyl carbamate)—more stable and hypnotic. Hydrazine, NH_2-NH_2 , is far more toxic than NH_3 , but the tetra- and penta-methylenediamines are non-toxic.

The entry of the amino group into the benzene nucleus forms the groundwork of a large number of antipyretics and analgesics. The aromatic amines are less basic than ammonia. Aniline, like ammonia, produces convulsions, but like benzene it also causes paralysis of muscles and nerves, and if one of the hydrogen atoms of the NH_2 group be replaced by alkyl the convulsions disappear but the paralyzing action remains. If a hydrogen atom in the nucleus be replaced by a single atom, e.g., Br, the convulsive effect is retained, and if it is replaced by an alkyl group the effect is increased, but if a complex group, especially an acid group, e.g., SO_3H , enters the nucleus, the effect is lost, e.g., in aminobenzene-sulphonic acid, $C_6H_4\cdot NH_2\cdot SO_3H$. All these derivatives, e.g., aniline, have a toxic action on the blood, forming methæmoglobin. As a rule, aromatic derivatives of NH_3 lower body temperature.

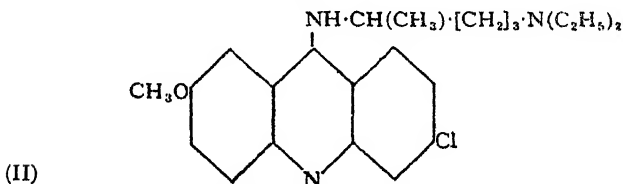
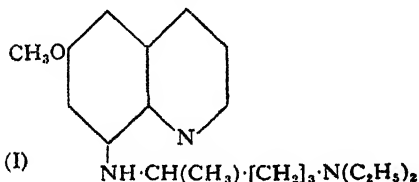
Alkaloids. No general clue to physiological effects is possible since in most cases the whole molecule appears to be essential. Most alkaloids possess valuable and potent physiological actions often accompanied by dangerous and unwanted side effects, as witness the habit-forming characteristics of morphine and cocaine. For this reason the efforts to synthesise compounds retaining the physiological effect of many alkaloids, but without the unwanted reactions, have not only contributed new and important drugs but have been instrumental in widening the boundaries of knowledge of chemotherapy. The following summary contains many of the recent introductions of synthetic

"chemical improvements" of certain alkaloids which have been produced by work along these lines.

(See also J. A. Aeschlimann, *J. Soc. chem. Ind., Lond.*, 1935, 136T.)

Quinine. This alkaloid has both antipyretic and antimalarial activity. Consideration of the activity of the various cinchona alkaloids as antimalarials indicates that neither the methoxy group nor the double bond present in quinine is essential. On the other hand the alteration of the secondary alcoholic group in quinine by oxidation or reduction destroys the activity. The synthetic antimalarials that have been introduced bear little relationship to quinine in structure and hence it appears that there are two essentials necessary for antimalarial activity: the presence of a quinoline nucleus and a centre of strong basicity.

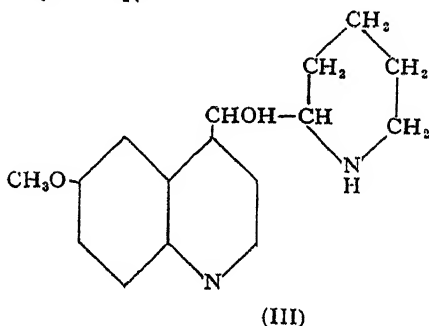
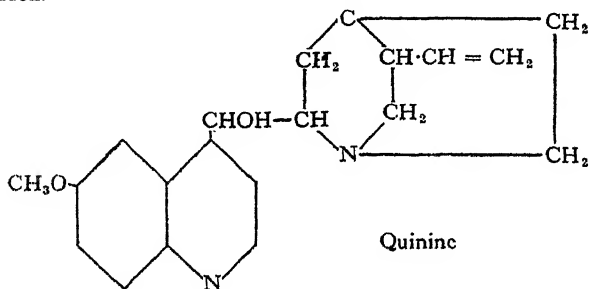
The first synthetic antimalarial was pamaquin (I), introduced in 1927 under the name of Plasmoguin. This substance acts at a different stage in the life history of the parasite from quinine and was obtained as a result of studies on the molecule of methylene blue which was known to possess some antimalarial activity. Mepacrine (II), introduced under the name of Atebrin, was the result of the introduction of the basic side chain of pamaquin into other nuclei. Mepacrine acts similarly to quinine.



A large number of compounds similar to the above types have been produced in recent years (Pyman, *Chem. Ind. Rev.*, 1930, 758; Kernack, *J. chem. Soc.*, 1931, 3089; Mike and Robinson, *J. chem. Soc.*, 1933, 1467; Madison and Strukow, *Arch. Pharm., Berl.*, 1933, 271, 359; 1934, 272, 74) from which it appears that the 6-hydroxy-derivative instead of the alkoxy-derivatives are worthy of attention.

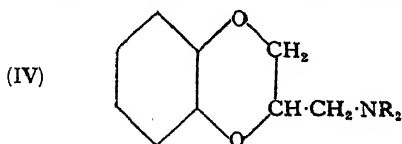
Little success has been achieved with synthetic antimalarials closely related to the quinine molecule. The first substance of

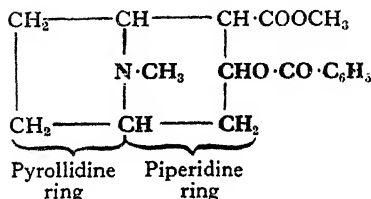
this nature possessing some activity, obtained by Ainley and King (*Proc. roy. Soc.*, 1938, 125, 60), was 4-(6-methoxyquinolyl)- α -piperidylcarbinol (III). It had about the same M.T.D. as quinine but was about one-half as effective in terms of the therapeutic index.



It is remarkable that N-alkyl derivatives of (III) possess no activity in spite of the fact that both nitrogens in quinine are tertiary.

Ergot Alkaloids. The constitution of the ergot alkaloids still remains unknown, and the problem becomes ever wider by the isolation of new alkaloids. However, Fourné and collaborators have found that the benzodioxan derivative (IV) possesses many of the characteristic actions of the ergot alkaloids.



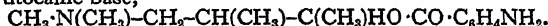


None as yet has been prepared containing the pyrrolidine ring, but β -eucaine (the lactate of the base being known as benzamine lactate) reflects the piperidine nucleus. Many have been derived by imitating the amino-alcohol structure represented in bold type in the above formula and sufficient data is available to say that all amino-alcohols, if esterified with a certain range of acids, yield local anæsthetics. Compare the following:—

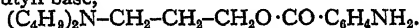
Structure in cocaine,



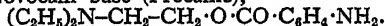
Tutocaine base,



Butyn base,



Novocain base (Procaine),



Stovaine base (Amylocaine),



The above examples illustrate the fact that the number of carbons between the amino and alcoholic groups need not be the same as in cocaine: Tutocaine most closely follows the cocaine plan but procaine and amylocaine depart from it. The acid used for esterification of the amino-alcohol is in many instances *p*-aminobenzoic acid as this appears to be more effective than benzoic acid. The simple esters of aminobenzoic acids and their derivatives, such as benzocaine (ethyl *p*-aminobenzoate), orthocaine (methylester of *p*-hydroxy-*m*-aminobenzoic acid) and propæsin (propyl *p*-aminobenzoate) indicate that the anæsthetic activity is retained in some measure by comparatively simple molecules.

General Anæsthetics. Since the introduction of ether by Long in 1842, closely followed by chloroform and nitrous oxide, few additional substances have been introduced as general anæsthetics. Practically the only other substance of importance is ethylene, which covers the same field as nitrous oxide.

Recently two new anæsthetics have been introduced, a preparation of vinyl ether (Vinesthene) and cyclopropane. Insufficient evidence is available concerning the latter but the former appears to induce anæsthesia very rapidly, allows quick recovery and

produces complete relaxation of the muscles. It has also found use in obstetrics. Another approach to this problem is illustrated by the introduction of bromethol (tribromoethyl alcohol dissolved in amylene hydrate) and the use of some of the newer barbiturates.

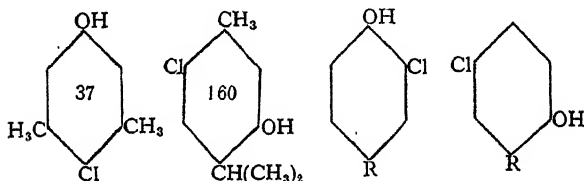
Barbiturates. 5 : 5'-Dialkyl or aryl derivatives of barbituric acid (malonylurea) form potent hypnotics. The number of carbon atoms in groups occupying this position should be between four and eight, one group being aliphatic. The use of some of the newer derivatives for producing basal anaesthesia has stimulated interest in this group, but there appears no distinct division into those which can be used for this purpose and those which are only useful as hypnotics. The table below gives the constitution of the groupings occupying the 5 : 5'-positions in malonylurea in the more important members of this group.

Phemitone and hexobarbitone illustrate a new departure—the formation of such compounds from methylurea instead of from urea.

The open chain ureides are much less active and much less toxic than the barbiturates, e.g., carbromal.

| B.P. Name. | | | | Proprietary Name. |
|-------------------|--|--|---------------|-------------------|
| Barbitone .. | $-\text{C}_2\text{H}_5$ | $-\text{C}_2\text{H}_5$ | H | Veronal |
| Phenobarbitone .. | $-\text{C}_6\text{H}_5$ | $-\text{C}_6\text{H}_5$ | H | Luminal |
| Phemitone .. | $-\text{C}_2\text{H}_5$ | $-\text{C}_6\text{H}_5$ | CH_3 | Prominal |
| Hexobarbitone .. | $-\text{CH}_3$ | | CH_3 | Evipan |
| | $-\text{CH}_2 \cdot \text{CH} = \text{CH}_2$ | $-\text{CH}_2 \cdot \text{CH} = \text{CH}_2$ | H | Dial |
| | $-\text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_3$ | $-\text{CHBr} \cdot \text{CH} = \text{CH}_2$ | H | Pernocton |
| | $-\text{C}_2\text{H}_5$ | | H | Phanodorm |
| | $-\text{C}_2\text{H}_5$ | $-\text{CH}_2 \cdot \text{CH} \cdot \text{CH}_2 \cdot \text{CH}_3$ | H | Nembutal |
| | $-\text{C}_2\text{H}_5$ | | H | Amytal |

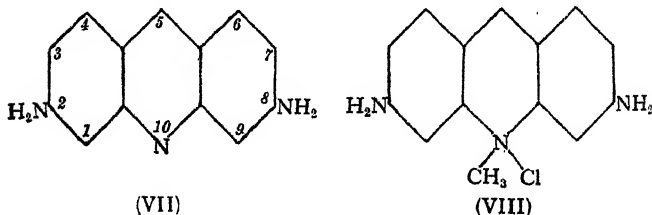
Bactericides. The cresols are approximately $2\frac{1}{2}$ times as active as phenol whilst with thymol the figure reaches 25. Toxicity is reduced with increase of molecular weight. Johnson and Lane (1921) determined the phenol coefficients for a series of 4-alkylresorcinols which increase up to the hexyl derivative (50) and then decrease (Dohme, Cox and Millar, 1926). Coulthard, Marshall and Pyman studied the variation of phenol coefficient with increase in the *n*-alkyl-side chain in the 4-*n*-alkylphenols, 4-*n*-alkylguaiacols and 4-*n*-alkyl-*m*-cresols. The *n*-amyl derivative was always the most active and the alkylcresols were all more active than the alkylphenols whilst the alkylguaiacols occupy an intermediate position. 4-*n*-Amyl-*m*-cresol exhibited a phenol coefficient of 280 and had about half the toxicity of hexylresorcinol. Attention has also been directed to the chloro derivatives of cresols and xlenols by the success of certain proprietary disinfectants, e.g.,



The number in the ring is the phenol coefficient. In the series represented by the two latter formulæ R must be butyl and amyl respectively to obtain greatest activity.

All phenol coefficient figures must be regarded with reserve as they refer only to one organism and to a particular set of experimental conditions. The figures may vary widely with different bacteria, but they are useful in providing a rough classification of compounds belonging to closely related series.

Derivatives of 2:8-diamino-acridine (VII) occupy a special position in the treatment of wounds as they are not inactivated in presence of serum. Their introduction is mainly due to the work of Browning in 1917.



Proflavine is the sulphate of (VII); acriflavine is a mixture of the hydrochlorides of (VII) and (VIII); whilst euflavine is a mixture of (VII) and (VIII). Albert and Linnell (1935-1938) prepared all the possible monoaminoacridines and diaminoacridines.

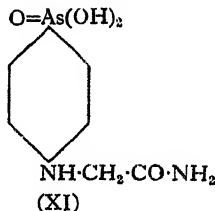
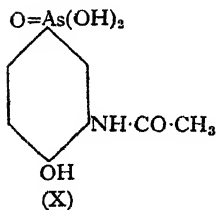
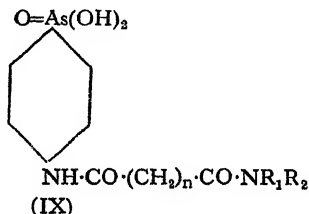
It was found that all compounds containing a 1-amino substituent were inactive and showed no fluorescence in solution. Amino groups in positions 2, 3, 4 and 5 increased the antiseptic activity of the acridine molecules, but toxicities and bacteriostatic properties were not parallel. 2:7-Diaminoacridine was found to be the best of this series of compounds and its toxicity, compared with the commercially available flavines, was 2:7-diaminoacridine, 1; proflavine, 2.5; acriflavine, 10. Later work of Falconer and Russell (*Proc. R. Soc. Med.*, 1940, 33, 494) has centred attention on 2:7-diaminoacridine as the best available antiseptic for the treatment of injuries to the living brain, whilst Manifold (*ibid.*, 1940, 33, 498; also *Brit. med. J.*, 1940, 631) has shown that it is much less toxic to brain tissue than acriflavine or proflavine. Linnell and collaborators have extended the exploration of the acridine nucleus to derivatives of dihydroacridine (the acridanes), the chloro-amino derivatives and the chloroalkoxy compounds (see *Quart. J. Pharm.*, 1938, 240; 1940, 162; 1942, 31; *J. chem. Soc.*, 1942, 377).

Organic Arsenicals. Bunsen first drew attention to the fact that cacodylic acid was almost non-toxic but Armand Gautier was the first to introduce organic arsenic compounds into medicine.

An aliphatic derivative containing arsenic, Arrhenal, $\text{CH}_3\text{AsO}(\text{ONa})_2$, is used as a general tonic but such substances are without action upon spirilla and trypanosomes whereas the aromatic compounds have a pronounced effect. Atoxyl, *p*-amino-arsonic acid, was used in control of sleeping sickness, of which the causal agent is *Trypanosoma gambiense*, although *in vitro* it exhibits no action against trypanosomes. Ehrlich supposed that it was reduced in the living organism and that the product was the actual agent in the trypanocidal action. Although this view has not been entirely substantiated it led to the introduction of Salvarsan (arsphenamine) and its derivatives neoarsphenamine and sulpharsphenamine, in which the arsenic is in the trivalent condition and which possess a much more satisfactory gap between the curative and the toxic doses. Arsenophenylglycine, another compound of trivalent arsenic, is very active in trypanosomiasis but has no action in spirillosis, whilst the reverse holds true for arsphenamine. The superiority of trivalent arsenic compounds has not been substantiated as tryparsamide, sodium *N*-phenylglycineamide-*p*-arsonate, and acetarsol, 3-acetylamin-4-hydroxyphenylarsonic acid, both contain the arsenic in the pentavalent condition: these compounds have the great advantage of being well defined crystalline identities which may be completely standardised by chemical analysis whereas the arsphen-

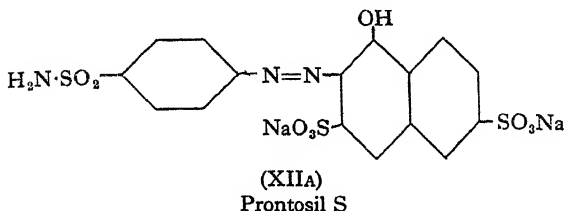
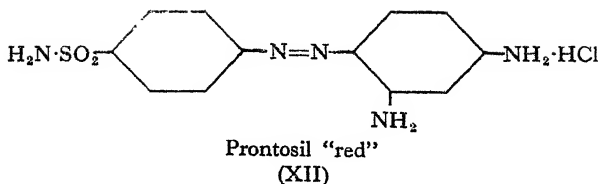
mines require a biological toxicity test in addition. Acetarsol was the first compound containing organic arsenic which exerted its effect when taken by mouth.

During the period 1931-1936 an extensive series of new organic arsenical compounds has been prepared at the Chemical Research Laboratory, Teddington, under the direction of Sir Gilbert Morgan, with the object of replacing or supplementing tryparsamide. The compounds possessed the general formula (IX) which combined in one structure the acyl and amide types represented by acetarsol (X) and tryparsamide (XI) respectively.

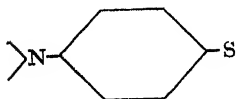


From over 100 active compounds, sodium succinylomethylamide-*p*-arsonate (Fig. IX, $n=2$, $\text{R}_1=\text{H}$, $\text{R}_2=\text{CH}_3$), now known as Neocryl, has been selected for human trials under the auspices of the Therapeutic Trials Committee of the Medical Research Council. It is still too early to give a final opinion as to the value of Neocryl but it appears to be much less toxic than tryparsamide.

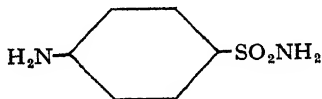
Streptococcicides. One of the most important developments of recent years has been the introduction of derivatives of *p*-aminobenzenesulphonamide (XV), following the work of Domagk (1935), as specifics in streptococcal infections. Domagk found that the dye, 4'-sulphamido-2:4-diaminoazobenzene, known as Prontosil (red) (XII) had the property of protecting mice against infection with virulent strains of hæmolytic streptococci. A soluble form (XIIa) was also introduced for parenteral administration: these substances have a potent action on streptococci localised in the body although they have little action *in vitro*.



It was established that the active fragment of these dyes was *p*-aminobenzenesulphonamide (XIV) and that this compound was formed in the animal organism by scission (Buttle, Gray and Stephenson, *Lancet*, i/1936, 1286; Kellner, *Thèse*, 1936, Paris; Fuller, *Lancet*, i/1937, 194). The results obtained with substances of this class against strepto-, pneumo- and staphylococcal diseases such as pneumonia and puerperal septicaemia, were often miraculous and hence it is not surprising that the subject has attracted large numbers of research workers. Up to 1940 some 3000 derivatives of *p*-aminobenzenesulphonamide had been prepared and tested. As a result of this work it is probably correct to say that a nitrogen *para* to a sulphur atom in a benzene ring (XIV) is essential for activity.



(XIII)

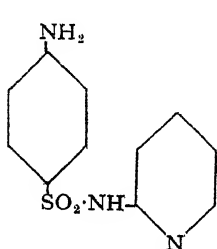


(XIV)

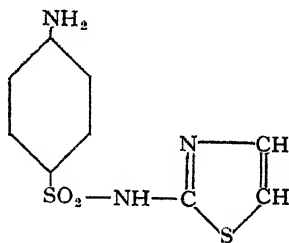
The related *ortho* and *meta* compounds of (XIV) are inactive. The hydrogens of the *p*-amino group may be replaced by a number of substituents without complete loss of activity; the hydrogens of the sulphonamide group may also be replaced by certain radicals whilst the activity is retained. The corresponding *p*-aminobenzenesulphonic acid, *p*-hydroxybenzenesulphonamide, and benzenesulphonamide itself, have little activity. Nuclear substitution of

the ring carrying the *p*-nitrogen and sulphur almost invariably causes complete inactivity, although other cyclic systems attached via substitution of the amino group can be varied in many ways.

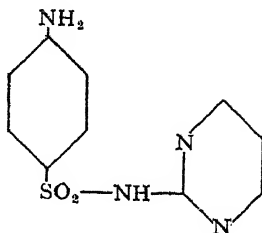
In addition to sulphanilamide (XIV) a comparatively small number of sulphonamide derivatives are in common use, of which the following are the most important:—



Sulphapyridine
(XV)



Sulphathiazole
(XVI)

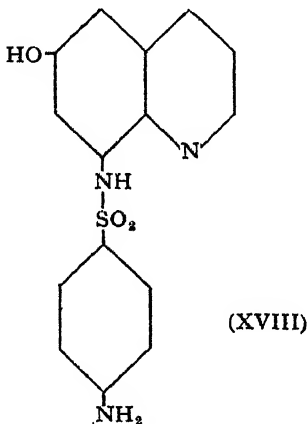


Sulphadiazine
(XVII)

Sulphanilamide, which is by far the cheapest substance of this group, should be used when contrary indications are absent. Sulphapyridine appears to have become established as the best derivative for certain types of pneumonia. The claims of sulphathiazole and sulphadiazine to attention are based upon lower toxicity, but they have been available for too short a time to make any firm statement about them. Sulphadiazine is stated to be particularly free from toxic effects and a high concentration may be maintained in the blood (*Brit. med. J.*, i/1942, 16).

The toxic effects of these derivatives are usually not of serious moment but can be very unpleasant and one of the major objectives of research is the reduction or elimination of this toxicity.

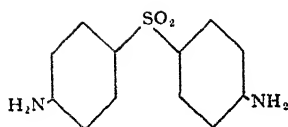
Sulphanilylguanidine, $p\text{-NH}_2\text{-C}_6\text{H}_4\text{-SO}_2\text{-NH}\cdot\text{C}(\text{:NH})\cdot\text{NH}_2$, the preparation of which has been described by Anderson and Cruickshank (*Brit. med. J.*, ii/1941, 497) is a compound which is said to be valuable as an intestinal antiseptic in the treatment of bacillary dysentery. The preparation of the compound (XVIII) is protected by patent (Brit. Pat. 531957) and antimalarial activity is claimed for it. If this is substantiated, it will extend the usefulness of the sulphonamides to another important field.



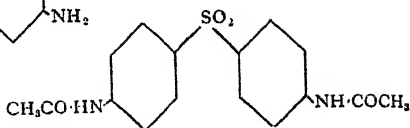
Little success has been encountered to date in introducing the sulphonamide group into other important chemical nuclei; for instance, the acridine derivatives so far obtained have been disappointing.

Future work on the sulphanilamide molecule has the dual objective of reducing toxicity and, by varying the molecule, extending its usefulness against other organisms.

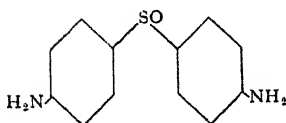
Sulphones. Certain sulphones, such as pp' -diaminodiphenylsulphone (XIX), have been found to possess a much greater activity than sulphanilamide, but, unfortunately, this was coupled with a greater toxicity. The sulphone molecule, however, appeared to be less susceptible to loss of activity by modification than sulphanilamide. For instance, the diacetyl derivative (XX) retained much of the activity of the parent body, but was much less toxic. Again, the corresponding sulfoxide (XXI) and sulphide (XXII) still possessed some activity.



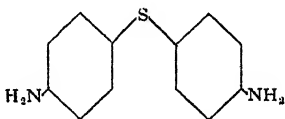
(XIX)



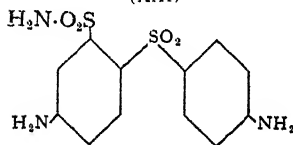
(XX)



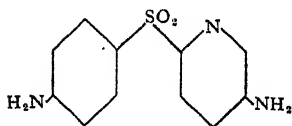
(XXI)



(XXII)



(XXIII)



(XXIV)

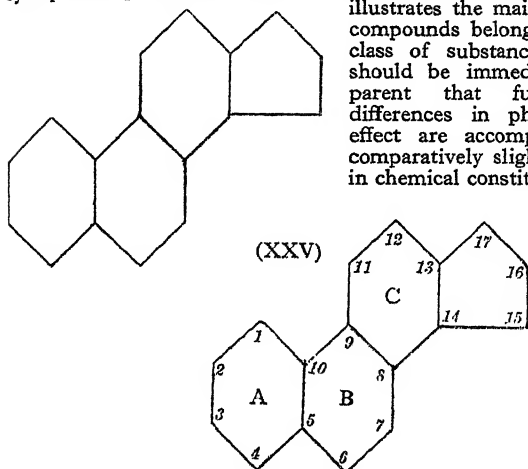
For these reasons this sulphone molecule may provide an even more fruitful source of new compounds than sulphanilamides. Roblin, Williams and Anderson (*J. Amer. chem. Soc.*, 1941, *63*, 1930; *Chem. Abstr. A.* 1942, *II*, 119) have prepared a series of compounds related to (XIX), two of which (XXIII, XXIV) were highly active against streptococcal and pneumococcal infections in mice, but less toxic than the type. The compound (XXIV) is most interesting when compared with sulphapyridine (XV). It may be that the introduction of the pyridine molecule into the sulphone field will yield as interesting results as it did amongst the sulphanilamides. Encouraging results have been obtained by Feldman (*Proc. Mayo Clin.*, 1941, 187; 1941, *III*, 125) in the treatment of tuberculosis in guinea-pigs with "promin," the sodium salt of the NN'-diglucosesulphonate of (XIX); its value in human infections is not yet established.

An excellent summary of the early work in this series will be found in the *Chemical Review* for 1940.

Sex Hormones. A remarkable instance of the changes in function following comparatively small changes in chemical

constitution is afforded by the natural substances based upon the cyclopentenophenanthrene skeleton (XXV). The table on p. 494,

illustrates the main types of compounds belonging to this class of substances, and it should be immediately apparent that fundamental differences in physiological effect are accompanied by comparatively slight changes in chemical constitution.



It will be noticed that throughout this series of substances, from cholesterol to testosterone there is a progressive shortening of the side chain at C_{17} . The main difference between progesterone and corticosterone appears to lie in the constitution of the C_{17} group. That the $-\text{CO}\cdot\text{CH}_2\text{OH}$ in corticosterone is important has recently been demonstrated by Linnell and Roushdi (*Quart. J. Pharm.*, 1939, 252; *ibid.*, 1941, 270; *Nature, Lond.*, i/1941, 595), who have produced two synthetic compounds containing this group which possessed biological activity similar to that of corticosterone.

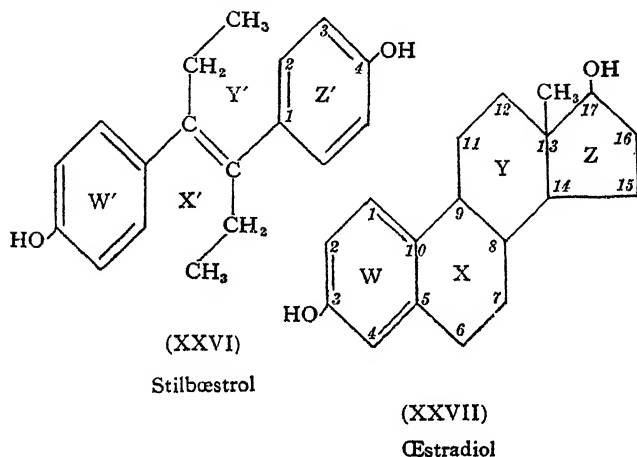
The main difference between the male and female sex hormones appears to be in the degree of unsaturation.

A considerable amount of work concerning the relationship between chemical constitution and physiological activity amongst the oestrogens, has been done in recent years. Varying oestrogenic activity (ranging from compounds showing small but definite activity to those 10,000,000 times more potent) has been found in derivatives of phenanthrene (*see* rings A, B, C), anthracene, diphenyl, diphenylmethane and stilbene.

Dodds, Goldberg, Lawson and Robinson (*Nature, Lond.*, i/1938, 247; cf. *Lancet*, ii/1938, 1389) produced 4:4'-dihydroxy- α : β -diethylstilbene (stilboestrol, XXVI), which was actually slightly more potent than the natural hormone. If figures XXVI and XXVII are observed an apparent similarity in molecular

| Type | Example | Double Bonds | Carbon atoms | | | | Other variations |
|-------------------|------------------|----------------------------|--------------|-----------------|-----------------|---|------------------|
| | | | 3 | 10 | 13 | 17 | |
| Sterol .. | Cholesterol .. | $\Delta^5(6)$.. | OH | CH ₃ | CH ₃ | -CH(CH ₃)·CH ₂ ·CH ₂ ·CH ₂ ·CH ₂ ·(CH ₃) ₂ | |
| | Ergosterol .. | $\Delta^5(6,7-8)22(23)$ | OH | CH ₃ | CH ₃ | -CH(CH ₃)·CH = CH·CH(CH ₃)·CH(CH ₃) ₂ | |
| Cardiac Glycoside | Strophanthidin | — | OH | -CHO | CH ₃ | — | 5-14-dihydroxy |
| Bile Acid .. | Lithocholic Acid | — | OH | CH ₃ | CH ₃ | — | — |
| | Progesterone .. | $\Delta^4(6)$.. | -O | CH ₃ | CH ₃ | -CO·CH ₃ | — |
| Steroid Hormones | Corticosterone | $\Delta^4(6)$.. | -O | CH ₃ | CH ₃ | -CO·CH ₂ ·OH | 11-hydroxy |
| | Testosterone .. | Δ^4 .. | -O | CH ₃ | CH ₃ | -OH | — |
| | Œstradiol .. | $\Delta^{1(2)-3(4)-5(16)}$ | -OH | — | CH ₃ | -OH | — |

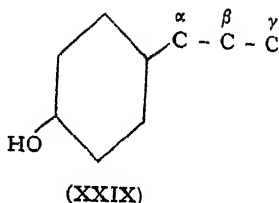
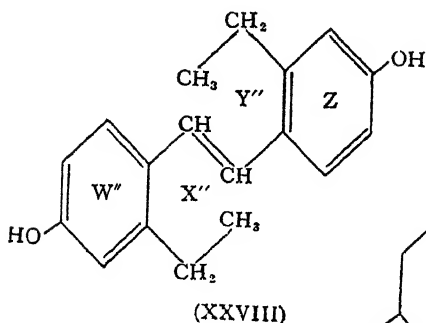
outline of this compound and œstradiol can be seen and it was suggested that this similarity might account for the remarkably high potency of the synthetic compound. Hexœstrol—in which the aliphatic double bond of stilbœstrol is reduced—is even slightly more active (see Docken and Spielman, *J. Amer. chem. Soc.*, 1940, 62, 2163, *Chem. Abstr. A*, 1940, 11, 342).



Rings W' and W are the same in each compound; rings X and Y may be considered to be simulated by the open chain structure X' Y'; ring Z' may be made by incorporating the angular methyl group into ring Z. On the other hand, the differences between the two structures are more fundamental: œstradiol has a condensed ring structure and this does not apply to stilbœstrol; the degree of saturation in the two compounds is widely different; both hydroxyls in stilbœstrol are phenolic, whereas in œstradiol one of them is a secondary alcohol. If this simulation of architectural plan is of moment, it appears that the 3 : 4'-dihydroxy analogue of stilbœstrol is closer to the structure of œstradiol than the 4 : 4'-dihydroxy compound as the C₁₇ hydroxyl is on the carbon "next door" to ring Y. Linnell and Sharma (*Quart. J. Pharm.*, 1941, 259) prepared this compound and found it to be much less active than stilbœstrol. It is probable, therefore, that simulation of architectural plan does not account for the high activity.

Linnell and Shaikhmahamud have prepared the compound (XXVIII) which simulates the structure of œstradiol to the same degree as stilbœstrol, but in a different manner. It will be noticed that the ethyl groups forming X'' and Y'' (XXVIII) are carried

by the nuclei W and Z and not by the α : β -carbons as in X' Y' (XXVI).



If the simulation theory were sound the compound would be expected to possess an activity of the order of stilbœstrol or œstradiol: if this is not true it should fall into place as a homologue of 4 : 4'-dihydroxystilbene. The compound was found to be active in doses of about 2 mg. (the figure for stilbœstrol is about 0.1 γ) and thus was placed in the latter category.

As a result of these studies Linnell and collaborators suggested that the molecular fragment of importance for œstrogen activity was the skeleton of 4-hydroxypropylbenzene (XXIX). Stilbœstrol can be considered as two such fragments joined through their α -carbon atoms; it may be discerned in the natural œstrogens in ring W plus carbons 9, 11, 12 (XXVII); it is not present in the molecule of (XXVIII). The above short summary of work in this field provides a good example of the methods used in addressing the problem of the relationship between chemical constitution and physiological effect.

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ACTION OF ACIDS ON THE COMMON METALS AND THEIR OXIDES

The reaction between acids and the common metals is a matter frequently arising and one concerning which information is not always available.

| SUBSTANCE | HYDROCHLORIC ACID Conc.* Sp. gr. 1.16 Dilute* Sp. gr. 1.048 | | SULPHURIC ACID Conc.* Sp. gr. 1.84 Dilute* Sp. gr. 1.069 | | NITRIC ACID Conc.* Sp. gr. 1.42 Dilute† Sp. gr. 1.057 | | REMARKS |
|---|--|--|---|---|--|---|--|
| Aluminium | Hot. Soluble. Forms AlCl_3 . | Easily soluble. Forms AlCl_3 . | Soluble. Forms $\text{Al}_2(\text{SO}_4)_3$. | Slowly attacked. | Soluble. Forms $\text{Al}(\text{NO}_3)_3$ and oxides of nitrogen. Scarcely attacked. | Soluble. Forms oxides of nitrogen. Slowly attacked. | Attacked by NaOH or KOH solutions. Soluble in cold acetic acid, more quickly in hot. |
| | Cold. Ditto. | Ditto. | Slightly attacked. | Unattacked. | Slowly soluble. Forms $\text{Al}(\text{NO}_3)_3$. Ditto. | Slowly soluble. Forms $\text{Al}(\text{NO}_3)_3$. Ditto. | <i>Ignited</i> (amorphous) oxide is unattacked by acids, except hot H_2SO_4 . |
| Antimony Oxide, (amorphous) As_2O_3 . | Hot. Slightly soluble. Forms AlCl_3 . Cold. Almost insoluble. | Slightly soluble. Forms AlCl_3 . Ditto. | Slightly soluble. Forms $\text{Al}_2(\text{SO}_4)_3$. Ditto. | Soluble. Forms $\text{Sb}_2(\text{SO}_4)_3$ and SO_3 . | Oxidised but not dissolved. | No action. | Aqua Regia dissolves, forming antimonious or antimonim chloride according to duration of action. |
| | Cold. No action. | No action. | Insoluble. | No action. | Practically no action. | No action. | |
| Antimonim Oxide, Sb_2O_3 . | Hot. Forms SbCl_3 . | Slightly soluble. | Slightly soluble. | Slightly soluble. | Practically insoluble. | Very slightly soluble. | Soluble in KOH and NaOH solutions. Insoluble in NH_4OH . |
| | Cold. Slowly soluble to form SbCl_3 . | No action. | Slightly soluble. | No action. | Ditto. | No action. | |

* = B.P.

† = B.P.C.

| SUBSTANCE | HYDROCHLORIC ACID Conc.* Dilute* Sp. gr. 1.16 Sp. gr. 1.048 | | SULPHURIC ACID Conc.* Dilute* Sp. gr. 1.84 Sp. gr. 1.069 | | NITRIC ACID Conc.* Dilute* Sp. gr. 1.42 Sp. gr. 1.057 | | REMARKS |
|--|---|--|--|---|---|---|---|
| Antimonious Oxide, Sb_2O_3 | Hot. | Soluble. Forms $SbCl_3$. | Soluble. Forms $SbOCl$ more or less according to proportion of acid. Slightly sol- uble. | Soluble. Very slightly soluble. | Forms Sb_2O_3 and Sb_2O_4 . | Slightly sol- uble. | Soluble in acetic, tartaric and benzoic acids, also in glycerin. |
| | Cold. | Slowly sol- uble. | | Slightly sol- uble. | | No action. | Easily soluble in Aqua Regia forming $SbCl_3$ or $SbCl_5$, according to duration of action. |
| Arsenic Arsenic Oxide, As_2O_3 | Hot. | Slowly sol- uble. Forms $AsCl_3$. | No action. | Soluble. As_2O_3 , no action. Forms As_2O_3 and SO_3 . No action. | Soluble. Forms H_3AsO_3 . Ditto. | Soluble. Forms H_3AsO_4 . No action. | Soluble in sodium hypo- chlorite solution. |
| | Cold. | Practically no action | Ditto. | No action. | | | Very soluble in water. |
| Arsenious Arsenious Oxide, As_2O_3 | Hot. | Soluble. Forms $AsCl_3$. | Soluble. Forms $AsCl_3$, and chlor- ine on pro- longed boil- ing. | Soluble. Forms $AsCl_3$. | Soluble. Forms H_3AsO_3 . Ditto. | Soluble. Forms H_3AsO_4 . No action. | Soluble in sodium hypo- chlorite solution. |
| | Cold. | Soluble with- out change. | Soluble. | Ditto. | Ditto. | Ditto. | Very soluble in water. |
| Arsenious Arsenious Oxide, As_2O_3 | Hot. | Soluble. Forms $AsCl_3$. | Soluble. Forms more or less $AsOCl$ ac- cording to propor- tions of acid. | Soluble. Slightly sol- uble. | Soluble. Forms H_3AsO_3 . | Soluble. Forms H_3AsO_4 . | Soluble in alkalis. |
| | Cold. | Ditto. | Slightly sol- uble. | Slowly sol- uble. | Ditto. | Slightly sol- uble. | |

| SUBSTANCE | HYDROCHLORIC ACID | | SULPHURIC ACID | | NITRIC ACID | | REMARKS |
|---|--|---|---|---|---|---|---|
| | Conc.* Sp. gr. 1.16 | Dilute* Sp. gr. 1.048 | Conc.* Sp. gr. 1.84 | Dilute* Sp. gr. 1.069 | Conc.* Sp. gr. 1.42 | Dilute† Sp. gr. 1.057 | |
| Bismuth | Hot. Scarcely acted on. | No action. | A slightly soluble basic sulphate formed and SO_4 . | No action. | Soluble. Forms $\text{Bi}(\text{NO}_3)_3$ and oxides of nitrogen. | Soluble. Forms $\text{Bi}(\text{NO}_3)_3$ or BiONO_3 according to quantity of acid. Scarcely acted on. | Aqua Regia converts into BiCl_3 . |
| | Cold. Insoluble. | Ditto. | Scarcely acted on. Schmidt says forms $\text{Bi}_2(\text{SO}_4)_3$. | Ditto. | Ditto. | | |
| Bismuth Oxide Bi_2O_3 . | Hot. Soluble. Forms BiCl_3 . | Soluble. | Slightly soluble. Forms $\text{Bi}_2(\text{SO}_4)_3$. Very slightly soluble. | Slightly soluble. Forms $\text{Bi}_2(\text{SO}_4)_3$. Very slightly soluble. | Soluble. Forms $\text{Bi}(\text{NO}_3)_3$. Ditto. | Soluble. Forms $\text{Bi}(\text{NO}_3)_3$. Ditto. | Soluble in strong hot NaOH solution. |
| | Cold. Ditto. | Ditto. | | | | | |
| Chromium (Reduced from CrCl_3 by Zn .) | Hot. Soluble. Forms CrCl_3 quickly oxidising to CrCl_4 . | Soluble. | Easily soluble. | Easily soluble. Forms CrSO_4 quickly oxidising to $\text{Cr}_2(\text{SO}_4)_3$. | Practically no action. | Insoluble. | Assumes the "passive" condition in contact with nitric acid and other oxidising agents. |
| | Cold. Ditto. | Very slightly soluble. | Insoluble. | Scarcely acted on. | Insoluble. | Insoluble. | |
| †Chromic Oxide, Cr_2O_3 (Green Amorphous). | Hot. Soluble. Forms CrCl_3 . Ditto. | Soluble. Forms CrCl_3 . Ditto. | Soluble. Forms $\text{Cr}_2(\text{SO}_4)_3$. Ditto. | Soluble. Forms $\text{Cr}_2(\text{SO}_4)_3$. Ditto. | Soluble. Forms $\text{Cr}(\text{NO}_3)_3$ (?) | Soluble. Forms $\text{Cr}(\text{NO}_3)_3$ (?) | Crystalline Cr_2O_3 is insoluble in all acids. |
| | Cold. Ditto. | | | | Ditto. | Ditto. | |

† Note.—By dissolving strongly heated chromic oxide in hot concentrated HNO_3 (Sp. gr. 1.4) a solution is obtained from which $\text{Cr}_2(\text{NO}_3)_6 \cdot 15\text{H}_2\text{O}$ crystallises on cooling. In dry air this loses $6\text{H}_2\text{O}$ with formation of $\text{Cr}_2(\text{NO}_3)_4 \cdot 9\text{H}_2\text{O}$.

| SUBSTANCE | HYDROCHLORIC ACID Conc.* Sp. gr. 1.16 | HYDROCHLORIC ACID Dilute* Sp. gr. 1.048 | SULPHURIC ACID Conc.* Sp. gr. 1.84 | SULPHURIC ACID Dilute* Sp. gr. 1.069 | NITRIC ACID Conc.* Sp. gr. 1.42 | NITRIC ACID Dilute† Sp. gr. 1.057 | REMARKS |
|---|--|--|---|--|--|--|--|
| Chromic Oxide, CrO_3 (Red). | Hot. Soluble. Forms CrCl_3 and chlorine. | Soluble without decomposition unless the solution be very concentrated | Soluble. Forms $\text{Cr}_2(\text{SO}_4)_3$ and oxygen. | Soluble without decomposition. | Soluble without decomposition. | Soluble without decomposition. | Very soluble in water to form H_2CrO_4 . |
| | Cold. Ditto. | Soluble without decomposition. | Soluble without decomposition. | Ditto. | Ditto. | Ditto. | |
| Cobalt Cobalt (ous) Oxide. | Hot. Soluble. Forms CoCl_2 . | Soluble. Forms CoCl_2 . | Attacked. Forms CoSO_4 and SO_2 . | Soluble. Forms CoSO_4 | Soluble. Forms $\text{Co}(\text{NO}_3)_2$ and oxides of nitrogen. | Soluble. Forms $\text{Co}(\text{NO}_3)_2$ and oxides of nitrogen. | |
| | Cold. Ditto. | Ditto. | Unattacked. | Ditto. | Ditto. | Ditto. | |
| | Hot. Soluble. Forms CoCl_2 . | Soluble. Forms CoCl_2 . | Soluble. Forms CoSO_4 . | Not attacked. | Soluble. Forms $\text{Co}(\text{NO}_3)_2$. | Soluble. Forms $\text{Co}(\text{NO}_3)_2$. | |
| | Cold. Ditto. | Ditto. | Ditto. | Ditto. | Ditto. | No action. | |
| Copper | Hot. Very slowly soluble. Forms Cu_2Cl_2 (in contact with the air). | Very slightly soluble. | Slowly soluble. Forms CuSO_4 , CuS and SO_2 . | Not attacked. | Soluble. Forms $\text{Cu}(\text{NO}_3)_2$ and oxides of nitrogen. | Soluble. Forms $\text{Cu}(\text{NO}_3)_2$ and oxides of nitrogen. | Slowly soluble in concentrated solutions of caustic alkalis. |
| | Cold. Not attacked. | Not attacked. | Not attacked. | Not attacked. | Ditto. | Scarcely attacked. | |

| SUBSTANCE | HYDROCHLORIC ACID Conc.* Sp. gr. 1.16 | Dilute* Sp. gr. 1.048 | SULPHURIC ACID Conc.* Sp. gr. 1.84 | Dilute* Sp. gr. 1.069 | NITRIC ACID Conc.* Sp. gr. 1.42 | Dilute† Sp. gr. 1.057 | REMARKS |
|--|--|---|--|---|--|--|--|
| Copper (-ic) Oxide, (Black), CuO. | Hot. Soluble. Forms CuCl ₂ . Cold. Ditto. | Soluble. Forms CuCl ₂ . Ditto. | Soluble. Forms CuSO ₄ . Slightly sol- uble. | Soluble. Forms CuSO ₄ . Ditto. | Soluble. Forms Cu(NO ₃) ₂ . Ditto. | Soluble. Forms Cu(NO ₃) ₂ . Ditto. | Slowly soluble in hot concentrated solutions of caustic alkalis. |
| Copper (ous) Oxide, (Red) Cu ₂ O. | Hot. Soluble. Forms Cu ₂ Cl ₂ . Cold. Forms CuCl ₂ and Copper. | Forms Cu ₂ Cl ₂ . Forms CuCl ₂ and Copper. | Soluble. Forms CuSO ₄ and SO ₂ . Forms CuSO ₄ and Copper. | Forms CuSO ₄ and copper. Ditto. | Soluble. Forms Cu(NO ₃) ₂ and oxides of nitrogen. Ditto. | Soluble. Forms Cu(NO ₃) ₂ and oxides of nitrogen. Slightly soluble. | Ditto. |
| Gold | Hot. Not attacked. Cold. Ditto. | Not attacked. Ditto. | Not attacked. Ditto. | Not attacked. Ditto. | Not attacked. Ditto. | Not attacked. Ditto. | Soluble in Aqua Regia to form AuCl ₃ . |
| Gold (ic) Oxide, Au ₂ O ₃ . | Hot. Slightly soluble. Cold. Ditto. | Slightly soluble. Ditto. | Slightly soluble. Ditto. | Slightly soluble. Ditto. | Soluble. Ditto. | Slightly soluble. Ditto. | Soluble in conc. KOH solution and KCN solution. |
| Iron | Hot. Soluble. Forms FeCl ₂ . Cold. Ditto. | Soluble. Forms FeCl ₂ . Ditto. | Soluble. Forms FeSO ₄ and SO ₂ . No action. | Soluble. Forms FeSO ₄ . Ditto. | Soluble. Forms Fe(NO ₃) ₂ and oxides of nitrogen. Rendered passive. | Soluble. Forms Fe(NO ₃) ₂ and oxides of nitrogen. Ditto. | |

| SUBSTANCE | HYDROCHLORIC ACID Conc.* Sp. gr. 1.16 Dilute* Sp. gr. 1.048 | | SULPHURIC ACID Conc.* Sp. gr. 1.84 Dilute* Sp. gr. 1.069 | | NITRIC ACID Conc.* Sp. gr. 1.42 Dilute† Sp. gr. 1.057 | | REMARKS |
|--|--|--|--|--|---|---|--|
| Iron (sic) Oxide, Fe_2O_3 . | Hot. Soluble. Forms Fe_2Cl_6 . | Soluble. Forms Fe_2Cl_6 . | Forms $\text{Fe}_2(\text{SO}_4)_3$ which dis- solves on dilution. Action slight. | Very slight action. | Very slight action. | Practically no action. | Strongly ignited oxide practically insoluble in all acids. |
| | Cold. Action slight. | Practically no action. | Action slight. | Practically no action. | Practically no action. | Ditto. | |
| Lead | Hot. Action slight. Forms PbCl_2 . | Action very slight. | Action vigorous. Forms PbSO_4 . | Action very slight. | Action slow. Forms $\text{Pb}(\text{NO}_3)_2$ and oxides of nitrogen. | Action vigorous. Forms $\text{Pb}(\text{NO}_3)_2$ and oxides of nitrogen. | Action greatly depends on the condition of the lead—whether sheet or finely divided, etc. |
| | Cold. Action very slight. | Ditto. | Action very slight. | Ditto. | Action slight. | Action slight. | |
| | Hot. Soluble. Forms PbCl_2 . | Soluble. Forms PbCl_2 . | Forms PbSO_4 . | Forms PbSO_4 . | Readily sol- uble. Forms $\text{Pb}(\text{NO}_3)_2$. | Easily sol- uble. Forms $\text{Pb}(\text{NO}_3)_2$. | Soluble in conc. KOH and NaOH solutions, easily in acetic acid. |
| | Cold. Ditto. | Ditto. | Ditto. | Ditto. | Ditto. | Ditto. | |
| Magnesium | Hot. Easily sol- uble. Forms MgCl_2 . | Easily sol- uble. Forms MgCl_2 . | Soluble. Forms MgSO_4 $\text{Mg}(\text{HSO}_4)_2$ and SO_4 . | Soluble. Forms MgSO_4 . | Soluble. Forms $\text{Mg}(\text{NO}_3)_2$ oxides of nitrogen, hydrogen and ammon. nitrate. | Soluble. Forms $\text{Mg}(\text{NO}_3)_2$ hydrogen and oxides of nitrogen. | Soluble in ammonium chloride solution. |
| | Cold. Ditto. | Ditto. | Action very slight. | Ditto. | Ditto. | Ditto. | |

| SUBSTANCE | HYDROCHLORIC ACID | | SULPHURIC ACID | | NITRIC ACID | | REMARKS |
|---|--|---|---|--|--|--|---|
| | Conc.* Sp. gr. 1.16 | Dilute* Sp. gr. 1.048 | Conc.* Sp. gr. 1.84 | Dilute* Sp. gr. 1.069 | Conc.* Sp. gr. 1.42 | Dilute† Sp. gr. 1.057 | |
| Magnesium Oxide, MgO. | Hot. Readily sol- uble. Forms MgCl ₂ . | Readily sol- uble. Forms MgCl ₂ . | Readily sol- uble. Forms MgSO ₄ and Mg(HSO ₄) ₂ . | Readily sol- uble. Forms MgSO ₄ | Readily sol- uble. Forms Mg(NO ₃) ₂ . | Readily sol- uble. Forms Mg(NO ₃) ₂ . | Soluble in ammonium salts, also in organic acids. |
| | Cold. Ditto. | Ditto. | Ditto. | Ditto. | Ditto. | Ditto. | |
| Manganese Dioxide, MnO ₂ . | Hot. Easily sol- uble. Forms MnCl ₂ . | Easily sol- uble. Forms MnCl ₂ . | Soluble. Forms MnSO ₄ and SO ₂ . | Easily sol- uble. Forms MnSO ₄ . | Easily sol- uble. Forms Mn(NO ₃) ₂ and oxides of nitrogen. Ditto. | Easily sol- uble. Forms Mn(NO ₃) ₂ and oxides of nitrogen. Ditto. | MnO ₂ is more soluble in diluted sulphuric acid in presence of easily oxidisable bodies (FeSO ₄ , sugar, etc.), with formation of MnSO ₄ and O ₂ , the O ₂ then oxidises the sub- stances in question. |
| | Cold. Ditto. | Ditto. | Action slight. | Ditto. | Action very slight. | Action very slight. | |
| | Hot. Soluble. Forms MnCl ₂ and chlorine. | Soluble. Forms MnCl ₂ and chlorine. | Action slight. Forms MnSO ₄ and oxygen at 200° for Mn ₂ (SO ₄) ₃ at 100°— Schmidt. | Action very slight. Forms MnSO ₄ and oxygen. | Action very slight. | Action very slight. | |
| | Cold. Ditto. | Action slight. | Practically no action. | No action. | No action. | No action. | |

| SUBSTANCE | HYDROCHLORIC ACID | |
|---|---|---|
| | Conc.* Sp. gr. 1.16 | Dilute* Sp. gr. 1.048 |
| Mercury | Hot. No action. | No action. |
| | Cold. Ditto. | Ditto. |
| Mercury (-ic) Oxide, (Yellow or red variety), HgO . | Hot. Soluble. Forms HgCl_2 . Cold. Ditto. | Soluble. Forms HgCl_2 . Ditto. |
| Nickel | Hot. Soluble. Forms NiCl_2 . | Very slowly soluble. Forms NiCl_2 . |
| | Cold. Ditto. | Ditto. |
| Nickel (-ous) Oxide, NiO . | Hot. Soluble. Forms NiCl_2 . Cold. Ditto. | Soluble. Forms NiCl_2 . Ditto. |

| SUBSTANCE | HYDROCHLORIC ACID | |
|---|--|---|
| | Conc.* Sp. gr. 1.16 | Dilute* Sp. gr. 1.048 |
| Nickel (-ic) Oxide, Ni_2O_3 . | Hot. Soluble. Forms NiCl_2 and oxygen. Cold. Ditto. | Soluble. Forms NiCl_2 and oxygen. Ditto. |
| Platinum | Hot. No action. Cold. Ditto. | No action. Ditto. |
| Silver | Hot. Practically no action. | Practically no action. |
| Silver Oxide, Ag_2O . | Cold. Ditto. | Ditto. |
| | Hot. Forms AgCl . Cold. Ditto. | Forms AgCl . Ditto. |
| Tin | Hot. Soluble. Forms SnCl_2 . | Soluble. Forms SnCl_2 . |
| | Cold. Soluble. Forms SnCl_2 . | Practically no action. |

| SULPHURIC ACID Conc.* Dilute* Sp. gr. 1.84 Sp. gr. 1.069 | | NITRIC ACID Conc.* Dilute† Sp. gr. 1.42 Sp. gr. 1.057 | | REMARKS |
|---|--|---|---|---|
| Forms HgSO_4 , SO_2 , and Hg_2SO_4 according to proportions and temperature. No action. | Practically no action. Ditto. | Soluble. Forms $\text{Hg}(\text{NO}_3)_2$ and oxides of nitrogen. | Soluble. Forms $\text{Hg}(\text{NO}_3)_2$ and oxides of nitrogen. | |
| Soluble. Forms HgSO_4 . Ditto. | Soluble. Forms HgSO_4 . Ditto. | Soluble. Forms $\text{Hg}(\text{NO}_3)_2$ and some $\text{Hg}_2(\text{NO}_3)_2$ and oxides of nitrogen. | Very slightly soluble. Forms $\text{Hg}_2(\text{NO}_3)_2$. | Combines easily with organic acids when freshly precipitated. |
| Action slight. Forms NiSO_4 and SO_2 . | Very slowly soluble. Forms NiSO_4 . | Easily soluble. Forms $\text{Ni}(\text{NO}_3)_2$ and oxides of nitrogen. Rendered passive. | Easily soluble. Forms $\text{Ni}(\text{NO}_3)_2$ and oxides of nitrogen. Ditto. | Soluble in NH_4OH . |
| Practically no action. | Ditto. | Soluble. Forms $\text{Ni}(\text{NO}_3)_2$. Ditto. | Soluble. Forms $\text{Ni}(\text{NO}_3)_2$. Ditto. | |

| SULPHURIC ACID Conc.* Dilute* Sp. gr. 1.84 Sp. gr. 1.069 | | NITRIC ACID Conc.* Dilute† Sp. gr. 1.42 Sp. gr. 1.057 | | REMARKS |
|--|---|---|--|---|
| Forms NiSO_4 . | Soluble. Forms NiSO_4 and oxygen. Ditto. | Soluble. Forms $\text{Ni}(\text{NO}_3)_2$ and oxygen. Ditto. | Soluble. Forms $\text{Ni}(\text{NO}_3)_2$ and oxygen. Ditto. | |
| Ditto. | | | | |
| No action. | No action. | No action. | No action. | Soluble in NH_4OH with evolution of nitrogen. |
| Ditto. | Ditto. | Ditto. | Ditto. | |
| Soluble. Forms Ag_2SO_4 and SO_2 . | Action very slight. | Soluble. Forms AgNO_3 and oxides of nitrogen. Ditto. | Soluble. Forms AgNO_3 and oxides of nitrogen. Action slight. | Finely divided silver is more responsive than compact silver to hydrochloric acid. |
| No action. | No action. | Soluble. Forms AgNO_3 . Ditto. | Soluble. Forms AgNO_3 . Ditto. | Soluble in NH_4OH and KCN solutions. |
| Soluble. Forms Ag_2SO_4 . Ditto. | Soluble. Forms Ag_2SO_4 . Slightly soluble. | | | |
| Dissolves, forming SnSO_4 (stannous sulphate) SO_2 and sulphur. Action slight. | Slowly soluble. Forms SnSO_4 . Practically no action. | Forms H_2SnO_3 (metastannic acid), oxides of nitrogen and NH_4NO_3 . Ditto. | Soluble. Forms H_2SnO_3 , $\text{Sn}(\text{NO}_3)_4$ and oxides of nitrogen & NH_4NO_3 . Soluble. Forms $\text{Sn}(\text{NO}_3)_2$, NH_4NO_3 and very little gas. | Soluble in hot concentrated NaOH or KOH solution. Forms stannates K_2SnO_3 or Na_2SnO_3 . Aqua Regia in excess dissolves to form stannic chloride, SnCl_4 . |

| SUBSTANCE | HYDROCHLORIC ACID Conc.* Sp. gr. 1.16 Dilute* Sp. gr. 1.048 | SULPHURIC ACID Conc.* Sp. gr. 1.84 Dilute* Sp. gr. 1.069 | NITRIC ACID Conc.* Sp. gr. 1.42 Dilute* Sp. gr. 1.057 | REMARKS |
|------------------------------------|---|--|--|---|
| Tin (-ic) Oxide, SnO_2 | Hot. No action. Cold. Ditto. | Slightly soluble. No action. | No action. | Slightly soluble in hot conc. NaOH or KOH solutions. |
| Tin (-ous) Oxide, SnO | Hot. Soluble. Forms SnCl_2 . Cold. Ditto. | Soluble. Forms SnSO_4 . Ditto. | Ditto. Forms SnO_2 and oxides of nitrogen. Ditto. Forms $\text{Sn(NO}_3)_2$. | Newth says solution in NaOH is known as sodium stannite. |
| Zinc | Hot. Soluble. Forms ZnCl_2 . Cold. Ditto. | Forms ZnSO_4 and SO_2 . Soluble. Forms ZnSO_4 and H_2S . Diluted, H_2S . Soluble. Forms ZnSO_4 and H_2 . | Soluble. Forms $\text{Zn(NO}_3)_2$. Soluble. Forms $\text{Zn(NO}_3)_2$, oxides of nitrogen and NH_4NO_3 . Soluble. | Soluble in hot concentrated KOH and NaOH solutions. |
| Zinc Oxide, ZnO | Hot. Soluble. Forms ZnCl_2 . Cold. Ditto. | Slightly soluble. Forms ZnSO_4 . Ditto. | Soluble. Forms $\text{Zn(NO}_3)_2$. Ditto. | Soluble in NH_4Cl , NaOH and KOH solutions. |

A SCHEME FOR THE RECOGNITION OF ORGANIC CHEMICAL SUBSTANCES USED IN THERAPEUTICS

The following scheme is intended to assist in the recognition of a number of organic chemicals, both natural and synthetic, used therapeutically. It frequently happens that the analyst is called upon to identify such substances, and without a systematic method of examination accurate results may be extremely difficult to obtain.

Before commencing the analysis it should be ascertained, for example by treatment with solvents, whether the sample is one compound or a mixture, and if the latter a separation by chemical or physical means should be effected. No general rule for the isolation of the constituents can be given, but fractional distillation, crystallisation and solution methods are usually used, and extraction from an acid or alkaline aqueous solution with an immiscible solvent is often helpful when one component has basic or acidic properties.

The Preliminary Tests are first carried out, and by comparison of the results with the tables, some idea of the nature of the substance may be obtained, enabling corroborative tests to be at once applied. If, however, no satisfactory evidence is obtained from the preliminary tests, it is determined whether the substance contains nitrogen, sulphur, halogens or phosphorus, a more complete classification for the purposes of identification being based on the elements present.

PRELIMINARY TESTS

1. The Action of Heat

A small quantity of the substance is heated on a piece of foil or porcelain and the odour, behaviour, and presence of any inorganic residue is noted.

| OBSERVATIONS | COMPOUNDS |
|---------------------------|--|
| ODOUR on heating: | Given by:— |
| (i) Very objectionable | Acriflavine, Benzocaine, Carbromal, Physostigmine, Yohimbine. |
| (ii) Garlic-like ... | Chloralamide, Disodium Methylarsonate, Neoarsphenamine, Sodium Aminarsonate, Sodium Cacodylate, Sulphonal, Thiosinamine. |
| (iii) "Burning sugar" ... | Citrates, Lactates, Mandelates, Tartrates, Sugars, Calcium Saccharate, Picrotoxin, Strophanthin. |

| OBSERVATIONS | COMPOUNDS |
|---|--|
| ODOUR on heating: | Given by:— |
| (iv) Resembling pyridine (or "burnt feathers") | The Purine Bases, Caffeine, etc., and their compounds. |
| (v) Phenolic | Amidopyrine, Apomorphine, Morphine, Cinchona alkaloids, Malachite Green, Phenazone Amylocaine Hydrochloride, Strychnine. |
| (vi) Aromatic | Inorganic Benzoates, Salicylates and Phenol-sulphonates, Phenol compounds. |
| (vii) "Sweetish" odour ... | Potassium Hydroxyquinoline Sulphate, Tribromophenol, Chlorocresol, Chloroxyleneol. |
| (viii) "Alcoholic" ... | The characteristic odour of Salicylic Acid on heating is obtained with all its organic derivatives. |
| (ix) Amine odour ... | Aconitine, Atropine, Cinchophen, Cocaine, Colchicine, Hyoscyne, Papaverine, Cinnamates, Benzoates, Hippurates. |
| (x) Odour of burning animal or nitrogenous matter | Allantoin, PicROTOXIN, Resorcinol, Saccharin, Ethylmorphine. |
| (xi) Acetous odour ... | Chlorbutol, Homatropine, Soluble Barbitone, Urethane. |
| (xii) Odour of geranium leaves | Amydracaine Hydrochloride, Amylocaine Hydrochloride, Apomorphine, Betaine, Colchicine, Ethylmorphine, Hexamine, Papaverine, Pilocarpine, Piperazine, Sodium Hippurate, Urea, Emetine Hydrochloride. |
| | Albumin Tannate, Bile Salts, Nuclein, Silver Proteinates. |
| | Atropine, Acetomenaphthone. |
| | Amphetamine. |
| APPEARANCE on heating: | |
| (i) Low-melting solids (below 100°) | Acetophenone, Stearic Acid, Benzocaine, Chloral Hydrate, Chlorbutol, Cocaine, Colchicine Salicyl., Coumarin, Guaiacol, Homatropine, Phenazone Salicylate, Physostigmine, Salol, Thiosinamine, Thymol, Tribromophenol, Urethane, Nikethamide, Cetyl Alcohol, Tribromoethyl Alcohol, Chlorocresol, Leptazol, Nupercaine. |
| (ii) Coloured fumes | Alkaloidal Periodides, Emet. Bism. Iodide, Iodoform, Thymol Iodide, Tetraiodopyrrol. |
| (a) of Iodine | Acriflavine, Chrysarobin, Colchicine, Fluorescein. |
| (b) Yellowish-brown | |
| (iii) Coloured residue— | |
| (a) Violet ... | Sulphanilamide. |
| (b) Brown (with evolution of sulphur dioxide) ... | |
| Brown (with odour of ammonia and mercaptan) ... | Sulphapyridine. |
| (iv) Yellow sparks | Sulphathiazole. |
| (v) Decomposes or volatilises without appreciable charring. | Gallic Acid, Alum. Aceto-tart., Zinc Phenol-sulphonate, Urea. |
| | Acetylsalicylic, Malic, Oxalic and Succinic Acids, Caffeine, Theobromine, Amphetamine, Phenacaine, Chloralamide, Naphthalene Tetra-chloride, Phenazone, Piperazine, Urea. |

If an inorganic residue is obtained on ignition the substance is probably the metallic salt of an organic acid or phenol, and the following compounds, classified according to the metal present, are frequently employed therapeutically. In most cases tests for the acidic radicle, which can usually be separated as the sodium salt by treatment of the substance with alkali, are described later.

| THE METAL PRESENT IN RESIDUE AFTER IGNITION | COMPOUNDS TO BE EXAMINED FOR:— |
|---|--|
| Aluminium | Aceto-tartrate. |
| Antimony | |
| (with Sodium) | Sodium Antimonytartrate, Stibophen. |
| (with Potassium) | Potassium Antimonytartrate. |
| Bismuth | |
| (with Sodium or Potassium or both) | Alkali Bismuthyl Tartrates, Benzoate, Citrate, Gallate, Oxyiodogallate, Naphthol, Tribromophenol, Salicylate, etc. |
| Calcium | Acetylsalicylate, Formate, Lactate, Gluconate, Levulinate, Glycerophosphate, Saccharate, Guaiacolsulphonate. |
| Iron | Ammon. Citrate, Tartrate, Oxalate, etc. Glycerophosphate, Peptonate, Valerianate. |
| Magnesium | Acetylsalicylate, Glycerophosphate, Ricinoleate. |
| Manganese | Butyrate, Glycerophosphate. |
| Mercury | Mersalyl, Phenylmercuric Nitrate. |
| Potassium | Acetate, Oxalate, Borotartrate, Citrate, Formate, |
| Silver | Colloidal, Proteinate, Nucleinate, etc. [etc. |
| Sodium | Indigo Carmine. Soluble Barbitone, Phenobarbitone, and Hexobarbitone, Phenytoin Soluble, Sodium Amytal, Iodoxyl, Suramin, Nembutal, Soluble Sulphanilamides, Mercurochrome, Neoarsphenamine, Acetate, Formate, Glycerophosphate, Salicylate, etc. Bile Salts. |
| Zinc | Oleate, Phenolsulphonate, Valerianate. |

2. The Action of Concentrated Sulphuric Acid

About 0.2 g. of the substance is treated with 1 ml. of sulphuric acid at first cold and then with heating.

| OBSERVATIONS | COMPOUNDS |
|---------------------------------|---|
| Cold Sulphuric Acid:— | |
| <i>Insoluble</i> | Saturated and aromatic hydrocarbons and their halogen derivatives:—Benzene, Xylene, etc. |
| Effervescence— | |
| (i) HCl, HBr, HI | From salts with organic bases, e.g.:— Acetyl- β -methylcholine Chloride, Aneurine Hydrochloride, Mepacrine Hydrochloride, Alkaloidal Hydrochlorides, Acriflavine, Betaine Hydrochloride, Procaine Hydrochloride, Amethocaine, etc. |
| (ii) Chlorine | Chloramine and Dichloramine-T. |
| Odour of Sulphur Dioxide | Neoarsphenamine, Sulphapyridine. |
| Coloration— | |
| (i) Deep red | With certain glycosides, e.g.:— Salicin, Amygdalin, Arbutin. Aloes, Acriflavine, Iodised Oils, Proflavine, Phenolphthalein, Santalol. |

| OBSERVATIONS | COMPOUNDS |
|--|--|
| <i>Coloration—</i> | |
| (ii) Light red ... | Nicotine, Physostigmine. |
| (iii) Green ... | Methylene Blue. |
| (iv) Blue ... | Strophanthin (changes to brown). Amyl Nitrite (changes to brown). |
| Hot Sulphuric Acid:— | |
| <i>Effervescence—</i> | |
| (i) Without charring | Formic Acid, Oxalic Acid, Betaine Hydrochloride, Urea. |
| (ii) With blackening | Carbohydrates, some glycosides, Hydroxyacids such as Citric, Tartaric, Lactic. |
| <i>Iodine evolved ...</i> | Apiol, Malachite Green. |
| <i>Pungent Vapours—</i> | Ethyl Iodide, Bismuth Oxyiodogallate, Emetine |
| Without effervescence and blackening ... | Bismuth Iodide, Thymol Iodide, Tetraiodopyrrol, Diodone, Iodoxyl. |
| <i>No blackening ...</i> | Acetic, Benzoic, Salicylic, Succinic, Acetylsalicylic Acids, Phenols and metallic derivatives, Brometone, Chlorbutol, Chloral Hydrate. |
| | Benzoic, Salicylic, Acetylsalicylic Acids and many of their compounds; Phenol. |
| | Allantoin (red soln.), Alloxan (yellow soln.), Amidopyrine, Amydicaine Hydrochloride, Chloralamide (chloral odour), Ethyl Bromide |
| | Fluorescein, Purine Bases. |

3. The Action of Alkali

To about 0.5 g. of substance, add strong sodium hydroxide solution and warm.

| OBSERVATIONS | COMPOUNDS |
|---|--|
| <i>Substance dissolves ...</i> | Acids, Amides, Barbiturates, Sulphanilamides, Phenols, Halogen deriv. of Phenols, some Esters, etc. |
| <i>Precipitate forms ...</i> | This may be hydroxide from metallic salt (which would be detected in Test 1). Organic Base from Salts (Morphine dissolves in excess). |
| <i>Odour of Ammonia ...</i> | From Ammonium Salts, Amides, Carbachol, Diphenan, Phemitone, Ureides, Urea compounds, Chloralamide. |
| <i>Odour of Amine ...</i> | From salts, e.g., Aniline Hydrochloride, Acetanilide, Methylacetanilide. |
| <i>Odour of Pyridine ...</i> | Nicotinic Acid. |
| <i>Coloration—</i> | |
| (i) Deep red... .. | Phenolphthalein. |
| (ii) Yellow cold turning red on warming | Many sugars. |
| <i>Fluorescence ...</i> | Dithranol, Fluorescein, Eosin. |

4. Reactions for Alkaloids

The solution should be tested for alkaloids by means of the usual reagents such as Mayer's reagent, gold chloride or picric acid. The table below may be useful in the identification of a common alkaloid (see page 514 *et seq.*)

In addition to the synthetic Cocaine substitutes the following bodies react in some cases like alkaloids:—

Amidopyrine, Phenazone, Piperazine, Piperidine, Potassium Hydroxyquinoline Sulphate, Pyridine, Quinoline, Thiosinamine, and some dyes such as Acriflavine.

The purine bases, Caffeine, Theobromine, Theophylline, do not respond to many of the usual tests.

5. Reaction with Fehling's Solution

It should be determined whether the substance reduces Fehling's before and after hydrolysis with dilute acid.

| | |
|---|--|
| Substances readily reducing Fehling's Solution before hydrolysis. | Monosaccharides: Dextrose, Lævulose, Mannose, etc. Disaccharides: Lactose, Maltose, etc. Fehling's is also reduced by some aldehydes, e.g., Chloral, and polyhydric phenols, e.g., Resorcinol, Pyrogallol, and by Amylocaine Hydrochloride, Camphoric Acid, Chloroform, Chloralamide. |
| Substances readily reducing Fehling's Solution only after hydrolysis. | Disaccharides: Sucrose. Glycosides: Aesculin, Salicin, etc. |

EXAMINATION FOR ELEMENTS

The compound should be examined systematically for non-metallic elements including nitrogen, sulphur, halogens and phosphorus. Lassaigne's method is usually satisfactory, and consists in adding about 0.1 g. of the substance to a small piece of clean molten sodium in a test-tube, and, after thorough heating, the mass is carefully treated with water and filtered.

Nitrogen

A portion of the above solution is tested for cyanide by heating with ferrous sulphate, making acid with hydrochloric acid and adding 1 drop of ferric chloride solution. A blue solution or precipitate of Prussian blue indicates the presence of nitrogen in the substance. Cyanamide, given by urea and derivatives, should also be tested for by means of silver nitrate, the silver salt being soluble in nitric acid but insoluble in ammonia. Sodium thiocyanate may be formed if the substance contains nitrogen and sulphur.

Sulphur

The formation of a deep violet colour on adding a small crystal of sodium nitroprusside to some of the solution from the sodium ignition indicates the presence of sulphur. The solution may also be tested for sulphide by means of lead acetate.

Halogens

These are identified in the usual way, after removal of hydrocyanic acid if necessary, by boiling some of the above solution with nitric acid.

Phosphorus

This element can be detected as phosphate after ignition of the substance with potassium carbonate and potassium nitrate.

It is now possible to decide to which of the following groups the unknown compound belongs:—

- GROUP I. *Organic compounds not containing halogens, nitrogen, sulphur or phosphorus.*
- GROUP II. *Compounds containing halogens* (and free from phosphorus, sulphur or nitrogen).
- GROUP III. *Compounds containing nitrogen* (and free from halogens, sulphur and phosphorus).
- GROUP IV. *Compounds containing sulphur* (and free from halogens, nitrogen and phosphorus).

GROUP V. *Compounds containing nitrogen and sulphur* (and free from halogens and phosphorus).

GROUP VI. *Compounds containing nitrogen and halogens* (and free from sulphur and phosphorus).

GROUP VII. *Compounds containing halogens, nitrogen and sulphur* (phosphorus absent).

GROUP VIII. *Compounds containing phosphorus.*

The distinguishing tests in each group, which should be carried out in the order given, together with a knowledge of the physical properties of the substance, should enable it to be identified with the aid of the special tests in the Corroborative Chart. When the groups contain a large number of compounds they are divided, as far as possible, according to their chemical type, which should enable any uncommon medicinal body, not mentioned in the scheme, to be classified.

GROUP I. Organic Compounds not containing Halogens, Nitrogen, Sulphur or Phosphorus.

1. Acids

By treatment with sodium carbonate or by an approximate titration, using N/1 sodium hydroxide and phenolphthalein, it should be determined whether the substance is an acid. A neutral solution of the sodium salt is then tested with ferric chloride and calcium chloride, the behaviour of some common acids being shown in the following table. The polyhydric phenols, such as pyrogallol, and also a few acids containing nitrogen, although not belonging to this group, are also included for convenience.

Table of The Common Acids

| REAGENT | OBSERVATIONS | ACIDS |
|-----------------|--|---|
| Ferric Chloride | <i>Purple Coloration.</i> | |
| | (i) Not discharged by acetic acid. | Salicylic Acid. Acetylsalicylic Acid also gives this on warming with the reagent. |
| | (ii) Discharged by acetic acid. | Phenol. |
| | <i>Yellow Coloration.</i> | Mandelic Acid. |
| | <i>Red Coloration.</i> | |
| | (i) Discharged by HCl to yellow colour. | Formic and Acetic Acids. |
| | (ii) Not discharged by HCl but by HgCl_2 . | Sulphocyanic Acid. |
| | (iii) Not discharged by HCl or HgCl_2 . | Meconic Acid. |
| | (iv) Blackened by excess of KOH solution. | Pyrogallol. |
| | <i>Coloured Precipitate.</i> | |
| | (i) Buff coloured. The addition of HCl gives white crystalline body. | Benzoic, Hippuric and Cinnamic Acids. |
| | (ii) Reddish-brown ppt. | |
| | (a) Giving clear solution with HCl. | Succinic and Phthalic Acids. |
| | (b) White crystals with HCl. | Uric Acid. |
| | (iii) Blue-black, giving brown solution with H_2SO_4 . | Gallic and Tannic Acids. |
| | (iv) Prussian blue, discharged by NaOH. | Ferrocyanic Acid. |
| | (v) Brown solution giving Prussian blue on adding SO_2 . | Ferricyanic Acid. |

| REAGENT | OBSERVATIONS | ACIDS |
|--|---|---|
| Calcium Chloride to cold solution. | (i) White precipitate soluble on boiling. (ii) White ppt. soluble in HCl but insoluble in acetic acid. (iii) Crystalline powder soluble in HCl and acetic acid. | Malonic Acid. Oxalic Acid. Tartaric, Fumaric Acids. |
| Calcium Chloride on boiling. | (i) White ppt. on adding 1 drop ammonia solution, and soluble in acetic acid. (ii) White ppt. on adding equal volume of alcohol. Soluble in acetic acid. (iii) Greyish-white ppt. Soluble in HCl to pinkish solution. | Citric Acid. Malic Acid. Tannic and Gallic Acids. |

Other acids which should be tested for are Cacodylic, Camphoric, Coumaric, Valerianic, Gluconic, Levulinic and Lactic. Oleic and Stearic acids have a characteristic appearance.

2. Phenols and Lactones

The presence of these compounds is indicated by solubility in sodium hydroxide solution but not in sodium carbonate.

PHENOLS.—Phenol, Thymol, α - and β -Naphthol, Cresol, Xylenol.

LACTONES, dissolving slowly in hot alkali and reprecipitated by acid:—Coumarin (has characteristic odour and gives yellow solution with caustic alkali), Santonin.

3. Aldehydes and Ketones

Aldehydes reduce ammoniacal silver nitrate and give colour with Schiff's reagent. Ketones and aldehydes form derivatives with phenylhydrazine and semicarbazide, the melting-points of which are useful for identification, and many give crystalline compounds with sodium bisulphite.

The following should be tested for:—Acetaldehyde, Formaldehyde, Paraformaldehyde, Benzaldehyde, Acetone, Acetophenone, Metaldehyde, Paraldehyde.

4. Esters

These compounds, when hydrolysed by alkali, yield an acid and an alcohol or phenol.

(i) Esters derived from alcohols usually have a pleasant odour.

Ethyl Acetate, Amyl Valerianate, Methyl Salicylate, Benzyl Benzoate and Succinate, Methyl and Propyl Hydroxybenzoates.

(ii) Esters derived from phenols and used therapeutically are often compounds of either Salicylic Acid or Guaiacol.

Salicyl Salicylate, Salol, Guaiacol Benzoate, Cinnamate, Carbonate, Camphorate, Salicylate, Valerianate.

5. Hydrocarbons

These are insoluble in cold sulphuric acid; aromatic compounds have characteristic odour, and must be identified by physical properties.

Benzene, Light Petroleum, Toluene, Xylene, Paraffin.

6. Mercury Compounds

The substance should be tested for mercury after destroying organic matter with concentrated sulphuric and nitric acids.

Common compounds are the Benzoate, Lactate, Oleate, Phenate, Salicylate, Succinate, Succinimide, Mercurochrome, Mersalyl, Phenylmercuric Nitrate.

7. Compounds Soluble in Water

CARBOHYDRATES (except Starch and Cellulose). *Aliphatic Alcohols, Glycosides* (moderately soluble), *Polyhydric Phenols*.

(i) Sugars and Glycosides.

These would have been indicated by Preliminary Test 5. Glycosides likely to be present are:—Digitalin, Digoxin, Digitoxin (not very soluble in water), Salicin, Phloridzin, Strophanthin.

(ii) Common Alcohols.

Methyl, Ethyl, Propyl and Isopropyl, Amylene Hydrate (camphoraceous odour).

(iii) Polyhydric Phenols.

Resorcinol, Pyrogallol.

8. Substances having Characteristic Physical Properties

The following members of this group can be recognised by properties such as odour and appearance.

| LIQUIDS | | SOLIDS | |
|---|---|-----------------------|--|
| SUBSTANCE | DISTINGUISHING PROPERTY | SUBSTANCE | DISTINGUISHING PROPERTY |
| Apiol (may, however, be crystalline) | Green liquid. Peculiar odour. | Aloes, Aloin | Yellowish-brown with characteristic odour. |
| Amyl Alcohol | Characteristic odour. | Coumarin | Fragrant odour. Sublimes at 100°. |
| Capsicin | Reddish-brown oily mass. | Podophyllin | Characteristic odour. |
| Resorcinol Monoacetate | Oily substance with slight acetic acid odour. | Acetophenone | Odour resembling almonds and jasmine. |
| Santalol | Characteristic odour. | Terpin Hydrate | Slightly aromatic odour. Sublimes at 100°. |

Test specially for the neutral compounds Podophyllotoxin, Cantharidin, Elaterin.

GROUP II. Compounds containing Halogens. (Nitrogen, Sulphur and Phosphorus being absent.)

The following are commonly occurring substances:—

CHLORINE COMPOUNDS.

Amylene Chloral, Butylchloral, Ethyl Chloride, Carbon Tetrachloride, Chlorbutol, Chloral Hydrate, Chloroform, Chlorocresol, Chloroxyleneol, Chlorobenzenes, Naphthalene Tetrachloride.

BROMINE COMPOUNDS.

Bromal Hydrate, Bromoform, Camphor Monobromide, Ethyl Bromide, Tribromophenol, Tribromoethyl Alcohol, Bromural.

IODINE COMPOUNDS.

Ethyl Iodide, Thymol Iodide, Thyroxin, Iodised oils.

GROUP III. Compounds containing Nitrogen. (Halogens, Sulphur and Phosphorus being absent.)**1. The Alkaloids**

Members of this class of compounds, including some synthetic cocaine substitutes, would be indicated by Preliminary Test 4.

In the following table most of the substances in general use are arranged according to their behaviour with certain reagents, and by testing with these

in the order named it is possible to determine rapidly in which group an unknown alkaloid occurs, when it is identified by physical properties and special reactions.

In each test about 2 ml. of a 1% solution of the alkaloidal salt, or saturated if less soluble, and a few drops of the reagent are used, an immediate precipitate only being noted. Before adding potassium ferrocyanide 5%, platinum chloride 5%, potassium chromate 5%, or picric acid, the solution is slightly acidified with dilute hydrochloric acid. Perchloric acid and N/10 potassium permanganate are added to the neutral solution.

It must be remembered that this scheme is intended only as a guide, and results must not be interpreted too rigidly, since uncommon alkaloids are not included, and also anomalous results might possibly occur with impure alkaloids or with any deviation from the above conditions.

| REAGENT | ALKALOIDS which give a distinct precipitate with the reagent | Other Alkaloids which give a distinct ppt., but which would be detected in previous groups |
|---|--|---|
| I. Potassium Ferrocyanide in slightly acid solution. | Apomorphine Emetine Berberine Papaverine Cinchonidine Quinidine Cinchonine Strychnine | |
| II. Perchloric Acid to neutral solution. | (i) <i>Immed. reducing</i> $KMnO_4$ Aconitine Hydrastine Veratrine (ii) <i>Not reducing</i> $KMnO_4$, <i>immed.</i> Cocaine | Berberine. Emetine. Papaverine. Strychnine. |
| III. Platinic Chloride in slightly acid solution. | Diamorphine Nicotine Quinine | Apomorphine. Berberine. Cinchonidine. Cinchonine. Emetine. Hydrastine. Papaverine. Quinidine. Strychnine. |
| IV. Potassium Chromate in slightly acid solution. | Yohimbine (If impure, yohimbine may precipitate in the potassium ferrocyanide group) | All the alkaloids previously mentioned give ppt. except Aconitine, and Nicotine. |
| V. Picric Acid in slightly acid solution. | (i) <i>Immed. reducing</i> $KMnO_4$ Codeine Morphine Ethylmorphine Narceine Gelsemine Eserine (ii) <i>Not reducing</i> $KMnO_4$, <i>immed.</i> Atropine Hyoscyamine Homatropine Sparteine Hyoscine | All the alkaloids previously mentioned give a ppt. with this reagent. |
| VI. Mayer's and Gold Chloride. | Coniine Pilocarpine Ephedrine | All the alkaloids in this table give ppt. |

COLOUR REACTIONS WITH FROEHDE'S REAGENT

A few drops of the reagent are added to a little of the dry alkaloid in a white dish, the colour being observed after a few minutes. Colorations, being due to reduction of the reagent, are also given by some non-alkaloidal substances, e.g., Salicin, Phloridzin, Colocynthin.

The following alkaloids give a distinct colour:—

| | |
|--|----------------------------|
| Apomorphine (bluish-green). | Morphine (deep red). |
| Berberine (dark greenish-brown). | Narceine (reddish-green). |
| Codeine (bluish-green). | Nicotine (red). |
| Diamorphine (red). | Papaverine (bluish-green). |
| Emetine, in presence of NaCl (green). | Quinidine (pale green). |
| Ethylmorphine (yellow, turning green). | Quinine (pale green). |
| Hydrastine (green). | Veratrine (red). |
| | Yohimbine (violet). |

Other alkaloidal reagents are the following:—

AMMONIACAL SILVER NITRATE. A mixture of equal volumes of 2% silver nitrate solution and dilute solution of ammonia.

AMMONIUM SULPHOMOLYBDATE. Froehde's Reagent. Ammonium molybdate, 1 g., in concentrated sulphuric acid, 100 ml.

ERDMANN'S REAGENT. Mix 6 drops of nitric acid (sp. gr., 1.25) with water, 100 ml., add 10 drops of this to 20 ml. of concentrated sulphuric acid.

KRAUT'S REAGENT. Dissolve 8 g. of bismuth nitrate crystals in 20 ml. of diluted nitric acid (30% w/w HNO₃), add a solution of 27.2 g. of potassium iodide in 50 ml. of water and dilute to 100 ml.

MANDELIN'S REAGENT. Sulphovanadic acid. A 1% solution of sodium vanadate in concentrated sulphuric acid.

MARMÉ'S REAGENT. Dissolve 3 g. of cadmium iodide in 18 ml. of water containing 6 g. of potassium iodide.

MERCURIC CHLORIDE SOLUTION. 1 in 20.

MERCURIC CHLORIDE-SODIUM CHLORIDE. Dissolve mercuric chloride 5 g. and sodium chloride 0.75 g. in water to 100 ml.

PLATINIC CHLORIDE. 1 in 20.

PHOSPHOTUNGSTIC ACID. Dissolve sodium tungstate, 100 g., and sodium phosphate, 70 g., in water, 500 ml., and acidify with nitric acid.

PHOSPHOMOLYBDIC ACID. Sonnenschein's Reagent. Consists of a solution of sodium phosphomolybdate in nitric acid, prepared by acidifying a warm solution (50° to 60°) of sodium phosphate with nitric acid, and adding an excess of ammonium molybdate solution. The yellow precipitate is separated, washed with water, then with nitric acid and dissolved in a hot solution of sodium carbonate (using as little as possible). The solution is evaporated to dryness and ignited at low red heat till all ammonium salts are volatilised, the residue moistened with nitric acid and again ignited. The product, consisting of sodium phosphomolybdate, is dissolved in ten times its weight of water, and nitric acid (sp. gr., 1.42) added until the precipitate at first produced redissolves.

TANNIC ACID. A solution of tannic acid, 1 g., in water, 8 ml., and alcohol, 1 ml.

WAGNER'S REAGENT. Iodine in potassium iodide. Iodine, 5 g.; potassium iodide, 10 g.; water, 100 ml. In using this reagent, e.g., in testing for complete extraction in alkaloidal assays, it should be noted that water saturated with ether and then acidified gives a precipitate of iodine on adding this reagent. The precipitate may be distinguished from that due to an alkaloid by adding water. If due to iodine, it will redissolve.

Ferro- and ferricyanides used to differentiate numerous alkaloids microscopically.—W. M. Cumming and D. G. Brown, *Pharm. J.*, ii/1925, 141.

For formulæ for preparation of Mayer's, and Dragendorff's Solution, *vide* pp. 208 and 108, Gold Chloride solution is used 1 in 20.

MICROCHEMICAL TESTS FOR ALKALOIDS.

Tentative or official tests are described in *Methods of Analysis (A.O.A.C.)*. The tests are carried out on a microscope slide using one drop of test solution and one drop of reagent, the effects produced being examined under a magnification of 100 to 150. The test solution is an approximately 1% aqueous solution of the alkaloidal salt or of the base dissolved with the aid of the minimum quantity of N/10 hydrochloric acid. The reagents are employed as 5% *w/v* aqueous solutions except in the case of the special reagents the formulæ for which are given. A table gives the results for a large number of alkaloids and closely related substances.

2. *Purine Bases*

These are weak bases reacting with only a few alkaloidal reagents, and are characterised by giving the murexide test, see Caffeine.

Caffeine, Theophylline, Theobromine and also their compounds with sodium salts such as Theobromine Sodium Salicylate. Uric Acid.

3. *Urea Compounds and Ureides*

Ureides are hydrolysed by strong potassium hydroxide, slowly in some cases, into the potassium salt of the acid and urea, the latter compound undergoing further decomposition giving potassium carbonate and evolving ammonia. (N.B.—Amides, e.g., Acetamide and Chloralamide, also yield ammonia on heating with alkali solution, but without formation of carbonate.) Note also precipitation with Millon's reagent, *v. Barbitone*, p. 533.

Test for Urea and Urethane (both readily soluble in water) and the ureides Alloxan, Allantoin, Allobarbitone, Barbitone, Hexobarbitone, Pamaquin, Phemitone, Phenobarbitone, Proponal, Soneryl, Veronal, etc.

4. *Amines*

Test for primary amines by the carbylamine reaction. Warm the substance with alkali and a little chloroform, and note any isocyanide odour.

This reaction is not given by primary amines capable of forming non-volatile salts, e.g., Aminophenols, Aminocarboxylic Acids, Aminosulphonic Acids.

If however, after heating, a sample is withdrawn on a glass rod and held in the current of air breathed out from the nose, the carbon dioxide combines with the alkali and liberates the volatile carbylamine derivative, the odour of which soon becomes noticeable.—*J. chem. Soc. Abstr.*, ii/1924, 430.

(i) *Aliphatic Primary Amines*

After solution of the substance in excess HCl and treatment with sodium nitrite these compounds yield an alcohol on heating, but they are rarely met with except as the amino-acids. Asparagine, for example, is converted into malic acid by nitrous acid.

(ii) *Aromatic Primary Amines*

These bodies form a diazonium compound with sodium nitrite and acid, which usually couples with an alkaline solution of β -naphthol forming dyes, and also gives a phenol on heating. Examine for Aniline, Benzocaine, Sodium Aminarsonate.

The following give reactions for a primary amine after hydrolysis:—
Acetanilide, Phenacetin.

(iii) *Secondary Amines*

Treatment with nitrous acid forms a nitroso compound which can be identified by Liebermann's reaction.

Methylacetanilide gives the secondary amine, methylaniline, after hydrolysis. The following compounds give colours with nitrous acid:—

| | | | |
|-------------|----------------|------------|---------------|
| Adrenaline | red colour. | Orthocaine | yellow colour |
| Amidopyrine | violet colour. | Phenazone | green colour. |

5. *Inorganic Matter Present*

Important compounds are those of Silver, e.g., Silver Proteinates, Colloidal Silver. The presence of inorganic matter in compounds of this group may denote a metallic cyanide, ferro- or ferricyanide.

Mercury Cyanide and Oxycyanide; Mersaly; Sodium compounds, Suebll, Barbiturates, etc.

6. Amino-Acids

These are neutral compounds usually soluble in water and insoluble in alcohol and ether.

Common substances are:—

Hippuric Acid.

Hydrolysed by HCl to benzoic acid and glycocoll.

Glycocoll (aminoacetic acid).

Gives deep blue colour with CuSO_4 due to copper glycocoll. FeCl_3 gives intense red colour discharged by acids.

Anthranilic Acid.

Heated with calcium oxide yields aniline. Treatment with nitrous acid and warming gives salicylic acid.

Betaine

Usually occurs as the hydrochloride which gives a very acid solution in water. Fusion with KOH gives trimethylamine.

Forms a periodide on adding a solution of iodine.

7. Esters of Nitrous and Nitric Acids

Amyl and Ethyl Nitrites have characteristic effect on inhaling. (See Vol. I, p. 160.)

Glyceryl Trinitrate, Mannityl Hexanitrate, and Erythrityl Tetranitrate are readily hydrolysed giving a nitrate. Being very explosive they usually occur only in solution or massed with an inert substance.

8. Nitro-compounds

Reduction in acid solution gives primary amines which can be identified as described above. Usually poisonous and not used medicinally.

GROUP IV. Compounds containing Sulphur. (Halogens, Nitrogen and Phosphorus being absent).

Phenolsulphonates of Zinc and Sodium, Sulphorcinates, Sulphonal, Thioresorcinol, Mesulphen.

GROUP V. Compounds containing Nitrogen and Sulphur (and free from Halogens and Phosphorus).

A solution of the substance should be tested for sulphate, and if present the organic base, which is probably alkaloidal, should be examined as described in Group III.

Other substances are:—Albumin Tannate, Amphetamine Sulphate, Bile Acids and Salts, Glycogen, Indigo Carmine, Neoarsphenamine, Potassium Hydroxyquinoline Sulphate, Proflavine, Saccharin, Sulphanilamide, Sulphapyridine, Sulphathiazole, Suramin, Thiosinamine.

GROUP VI. Compounds containing Nitrogen and Halogens (and free from Sulphur and Phosphorus).

A solution should be tested for ionised halogen by means of silver nitrate, which indicates the presence of a salt of an organic base. The latter if alkaloidal is described in Group III.

CHLORINE COMPOUNDS

Acriflavine, Arsphenamine, Betaine Hydrochloride, Chloralamide, Chloramine-T, Dichloramine-T, Fuchsine, Malachite Green, Mepacrine Hydrochloride, Carbachol.

Cocaine Substitutes, e.g., Amydracaine, Amylocaine, Procaine, etc., are mentioned in Group III.

BROMINE COMPOUNDS

Bromo-valerianyl-urea, Carbromal.

IODINE COMPOUNDS

Tetraiodopyrrol, Iodoxyl Diodone.

GROUP VII. Compounds containing Halogens, Nitrogen and Sulphur
(Phosphorus absent).

Methylene Blue, Thiosinamine Ethyl Iodide, Aneurine Hydrochloride, Chiniofon, Mepacrine Methanesulphonate.

GROUP VIII. Compounds containing Phosphorus.

Glycerophosphoric Acid and Salts, Alkaloidal Phosphates
Nuclein, Nucleinic Acid, Lecithin.

**Physical Constants of Value for the Recognition
of Organic Substances and used in the Table of
Corroborative Tests on page 520 et seq.**

Melting-Point. Substances which are readily reduced to a powder are dried at a temperature considerably below the melting-point. The melting-point is determined by the capillary tube method, using thermometers and apparatus which comply with specifications given in an appendix to the *B.P.* The thermometer and capillary tube are immersed in a suitable liquid, previously heated to a temperature 10° below the melting-point and the rate of rise of temperature is then carefully regulated to 2° per minute.

Boiling-Point. The boiling-point of a substance is defined in the *B.P.* as the range of temperature within which the whole or a specified portion of the substance distils. The determination is carried out on 100 ml. of the substance in a standard apparatus which is described in an appendix to the *B.P.* The heat is regulated to obtain a rate of distillation of 4 to 6 mls. per minute for liquids boiling below 150° and 2 to 4 mls. per minute for liquids boiling above 150° . The temperature is taken when the first drop of distillate falls from the condenser and subsequent readings are taken after specified amounts have distilled. Correction is made for the barometric pressure which is itself corrected for index error, temperature and gravity. For every 10 mm. above or below 760 mm. pressure, 0.4° is subtracted or added to the observed readings for most liquids. In the case of acetone the correction for 10 mm. is 0.38° and that for chloroform is 0.42° .

Refractive Index. The refractive index of a substance is determined in a refractometer at the specified temperature.

Optical Rotation. The optical rotation is determined in a polarimeter using a 1 dcm. tube containing the substance (if a liquid) or a solution of the substance of specified strength. The determination is made at 20° , sodium light being used. The specific rotation is calculated from the following formula:—

Specific Rotation = $\frac{\alpha \times 100}{l \times c}$, where α = observed rotation, l = length of tube in dcm., and c = concentration in g. per 100 ml. of solution examined.

Viscosity. The viscosity of a liquid is determined in a standard apparatus, specifications for which are given in an appendix to *B.P. Add. IV*. The determination is carried out at a specified temperature.

Specific Gravity. The specific gravity of liquids is determined at 15.5° unless otherwise stated.

CORROBORATIVE

The following table gives the physical constants and
It will be found useful when confirming results

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|---|-----------------------|-------------|-------------|-----------------|---------------------------------|---------------------------------------|
| 1 | Acetaldehyde | C_2H_4O | — | 21 | 0.80 | Miscible | Miscible |
| 2 | Acetanilide | C_8H_8ON | 113-115 | — | — | 210 | 4.2 |
| 3 | Acetannin | C_8H_8O | — | — | — | Almost insoluble | Almost insoluble |
| 4 | Acetarsol | C_8H_8ON , As | 240-250 | — | — | Almost insoluble | Insoluble |
| 5 | Acetomen- aphthone | C_8H_8O | 112-114 | — | — | Insoluble | Slightly soluble |
| 6 | Acetone | C_3H_6O | — | 56-58 | 0.796- 0.801 | Miscible | Miscible |
| 7 | Acetophenone | C_8H_8O | 18 | 200 | 1.035 | Insoluble | Miscible |
| 8 | Acetylami- dosalol | C_8H_8ON | 187 | — | — | Almost insoluble | Soluble |
| 9 | Acetylcholine Chloride | $C_8H_{10}ON$, Cl | — | — | — | Very soluble | Soluble |
| 10 | Acetyl-β- methyl- choline Chloride | $C_9H_{12}ON$, Cl | 172 | — | — | Soluble | Soluble |
| 11 | Acid Acetic | C_2H_4O | 15 | 117 | 1.055- 1.058 | Miscible | Miscible |
| 12 | Acid Acetyl- salicylic | C_9H_8O | 135-138 | — | — | 300 | 5 |

TESTS

characteristic reactions of many medicinal compounds.
obtained by the preceding general scheme of analysis.

| No. | SPECIAL TESTS |
|-----|---|
| 1 | Shaken with conc. sodium bisulphite solution gives crystalline addition-product, $\text{CH}_3\text{CH}(\text{OH})\cdot\text{SO}_3\text{Na}$, decomposed by acid or alkali. Combines with phenylhydrazine forming ethyldenenphenylhydrazone. Combines with NH_3 forming additive compound. |
| 2 | Hydrolysed by acid or alkali solution to aniline and acetic acid (or acetate), 0.1 g. boiled with 2 ml. of hydrochloric acid, then mixed with 3 ml. of phenol solution (1 in 20) and 5 ml. of saturated chlorinated lime solution, turns brownish-red changing to blue on adding ammonia (Indophenol Test). Heated with boric acid over a naked flame, gives yellow residue and sweet odour. Phenacetin gives yellow, phenazone a pink, and naphthalene an orange. 0.001 g. in 5 ml. water heated with NaOH and few drops chloroform gives phenyl isocyanide odour. |
| 3 | Shaking with ethyl alcohol and sulphuric acid gives odour of ethyl acetate. |
| 4 | A solution of 0.2 g. in 0.4 ml. of sodium hydroxide solution, diluted to 2 ml. with water, gives no ppt. in the cold with magnesium ammonio-sulphate solution but a white ppt. is produced on boiling. |
| 5 | Heated with H_2SO_4 conc. acetic acid is evolved with an odour of naphthalene. |
| 6 | Oxidation with potassium dichromate and sulphuric acid gives acetic and formic acids. Combines with chloroform in presence of alkali hydroxide to give acetone-chloroform (colourless insoluble crystals, m.p. 96°). 0.00001 ml. in 10 ml. of water warmed to 70° , 1 g. of potassium hydroxide added and then 10 drops of salicylaldehyde gives a purplish-red ring. |
| 7 | 0.01 g. in 5 ml. water with hydroxylamine hydrochlor. forms acetoxime, $\text{C}_6\text{H}_5\text{C}(\text{N}(\text{OH})\cdot\text{CH}_3$ (white ppt., m.p. 59°). This by boiling with dilute sulphuric acid in glacial acetic acid is converted into acetanilide (Beckmann's reaction), and then aniline (odour) and acetic acid. |
| 8 | Hydrolysed on warming with alkali into alkali salicylate and acetyl- <i>p</i> -aminophenol, the solution becoming blue. |
| 9 | Unstable in solution and readily hydrolysed by alkalis to choline. Aurichloride has m.p. 166° to 168° ; platinichloride has m.p. 256° to 257° . |
| 10 | Solution has bitter taste and readily hydrolysed by alkalis to methylcholine. |
| 11 | The nearly neutralised solution gives a deep red colour with ferric chloride, the liquid giving a reddish-brown ppt. on boiling, and a yellow colour on the addition of hydrochloric acid. |
| 12 | When boiled with alkali and the solution acidified, a white ppt. of salicylic acid is produced. |

| No. | SUBSTANCE | ELEMENTS | M. P. °C. | B. P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|-----------------------------|---------------|---|-----------------------|---|---------------------------------|---------------------------------------|
| 13 | Acid Agaric | C_7H_7O | 140 | — | — | Slight | 130 |
| 14 | Acid Amino- acetic | $C_2H_3O_2N$ | 233 (De- comp.) | — | — | 4 | Very slight |
| 15 | Acid Ascor- bic | C_6H_7O | 190-192 (De- comp.) | — | — | Readily | Soluble |
| 16 | Acid Benzoic | C_7H_7O | 121-122 | — | — | 450 | 3 |
| 17 | Acid Cacodylic | C_7H_7O, As | About 200 | — | — | 0.5 | 3.5 |
| 18 | Acid Camphoric | C_7H_7O | 185-187 | — | — | 160 | 1.5 |
| 19 | Acid Cinnamic | C_9H_7O | 132-135 | — | — | 3500 | Readily |
| 20 | Acid Citric | C_6H_7O | 153; becomes anhyd- rous at 135 | — | — | 0.6 | 1.5 |
| 21 | Acid Coumaric | C_9H_7O | 200 | — | — | 600 | About 12 |
| 22 | Acid Formic | C_1H_3O | — | 101 (when pure) | 1.245 (when pure) 1.06 (B.P.C.) | Miscible | Miscible |
| 23 | Acid Gallic | C_7H_7O | 220-230 (De- comp.) | — | — | 100 | 8 |
| 24 | Acid Glycero- phosphoric | $C_3H_5O_4P$ | — | — | About 1.1 (B.P.C.) | Miscible | Miscible |

| No. | SPECIAL TESTS |
|-----|---|
| 13 | Turns gelatinous and soapy on boiling with water. 0.00001 g. in 5 ml. water with 15 ml. sulphuric acid, cooled and a few drops of syrup added gives a purple in a few minutes. |
| 14 | The aqueous solution gives a red colour with ferric chloride solution. When boiled with copper oxide, filtered while hot and the solution cooled, bright blue needles of the copper salt are obtained. When treated with benzoyl chloride, hippuric acid (m.p. 187°) is obtained. |
| 15 | The aqueous solution decolorises solution of 2:6-dichlorophenol-indophenol, reduces Fehling's solution giving a yellowish ppt., and immediately reduces potassium permanganate solution. |
| 16 | 0.001 g. in 0.5 ml. water gives light buff ppt. with 1 drop of ferric chloride solution. 0.001 g. warmed with 3 drops of 25% formic acid and the solution neutralised with lime water, evaporated to dryness and the residue heated in ignition tube gives benzaldehyde odour. |
| 17 | 0.005 g. dissolved in 5 ml. water and a few drops hypophosphorous acid added gives a cacodyl odour in a few seconds. |
| 18 | Gives sublimate of anhydride, m.p. 217°, on heating. Neutralised solutions give a yellowish-brown ppt. with ferric chloride and a light blue ppt. with copper sulphate. |
| 19 | 0.0025 g. oxidised with potassium permanganate gives benzaldehyde odour. Can be reduced by sodium amalgam to hydrocinnamic acid (β -phenylpropionic acid). Detected in presence of benzoic acid by suspending in 5% uranium acetate solution and exposing to sunlight—in a few minutes the odour of benzaldehyde is evolved and a brown ppt. forms. |
| 20 | DENIGÉ'S TEST: Add mercuric sulphate solution to the citrate solution. On warming, this solution decolorises potassium permanganate, giving white ppt.; visible 0.001 g. in 5 ml. water. Tartaric acid does not react. A solution is boiled with a little potassium dichromate, and, after cooling, acetic acid and sodium nitroprusside added and then ammonia to form a layer. Acetone from the citric acid gives a violet-red colour at the surface of contact. |
| 21 | Melted with potassium hydroxide gives salicylate and acetate. Aqueous solutions of alkaline coumarates are fluorescent. |
| 22 | Gives a ppt. of mercurous chloride when warmed with mercuric chloride solution, and when warmed to 40° with mercuric oxide the filtrate gives a grey ppt. of mercury on boiling. Heated with conc. sulphuric acid, CO is evolved without charring. |
| 23 | Aqueous solution gives white ppt. with potassium antimonyltartrate solution. 0.000125 g. in 5 ml. water on adding lime water gives pink colour; 0.00025 g. gives purple; 0.0005 g. gives blue-grey ppt. 0.005 g. in 5 ml. water turns brown with sodium nitrite alone. Gives no ppt. with gelatin solution (<i>cf.</i> tannic acid). |
| 24 | 0.001 g. in 5 ml. water boiled 3 minutes with hydrochloric acid gives phosphoric acid and glycerin. |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|-------------------------|-----------|---------------------------|-----------------|-------------|---------------------------------|---------------------------------------|
| 25 | Acid Hippuric | C,H,O,N | 187 | — | — | Very slight | 30 |
| 26 | Acid Lactic | C,H,O | — | — | About 1·21 | Miscible | Miscible |
| 27 | Acid Malic | C,H,O | About 100 | — | — | 1 | 1·5 |
| 28 | Acid Mandelic | C,H,O | 118-119 | — | — | 8 | 2 |
| 29 | Acid Meconic | C,H,O | — | — | — | Slight | 48 |
| 30 | Acid Nicotinic | C,H,O,N | 234-237 | — | — | 75 | Readily soluble |
| 31 | Acid Nucleinic | C,H,O,N,P | — | — | — | Almost insoluble | Insoluble |
| 32 | Acid Oleic | C,H,O | — | 288 (15 mm.) | About 0·898 | Insoluble | Readily |
| 33 | Acid Oxalic | C,H,O | 98-100 | — | — | 9 | 8 |
| 34 | Acid Ricinoleic | C,H,O | Solidifies at about 4 | — | 0·945-0·948 | Insoluble | Soluble |
| 35 | Acid Salicylic | C,H,O | 158-159 | — | — | 500 | 3·5 |
| 36 | Acid Stearic | C,H,O | 50-55 (69·3 when pure) | — | — | Insoluble | About 25 |
| 37 | Acid Succinic | C,H,O | Not below 185 | — | — | 20 | 9 |
| 38 | Acid Sulphanilic | C,H,O,N,S | Decomposes at 280-300 | — | — | About 160 | Insoluble |

| No. | SPECIAL TESTS |
|-----|---|
| 25 | Boil 0.01 g. a few minutes with 1 ml. sodium hydroxide solution and neutralise with hydrochloric acid. Half this solution gives buff ppt. with ferric chloride (benzoate). Acidify the other, evaporate to dryness, extract the glycooll with ethyl acetate. This dissolves freshly pptd. copper carbonate giving a deep blue solution. Copper benzoate, if present, does not interfere and if necessary benzoic acid can be removed with ether in which glycooll is insoluble. |
| 26 | 1 g. warmed with 0.1 g. potassium permanganate evolves the odour of acetaldehyde. |
| 27 | Treated with potash and bromine, bromoform is formed. Heat 0.05 g. on a water-bath with 1 ml. of conc. sulphuric acid and a trace of β -naphthol; an intense yellow colour is produced. |
| 28 | Benzaldehyde is evolved on heating to 200°. |
| 29 | An aqueous solution with ferric chloride gives a red colour not discharged by hydrochloric acid or mercuric chloride (distinction from thiocyanate). |
| 30 | Pyridine evolved when heated with soda-lime. 6 ml. cyanogen bromide and 1 ml. 2.5% aqueous aniline to 2 ml. 0.1% solution in water gives golden-yellow colour; stronger solution will give red ppt. Yellow colour with excess of NaOH solution is due to presence of pyridyl-nitropyrazole. |
| 31 | Decomposes on boiling with dilute sulphuric acid; product yields phosphoric acid, carbohydrates and xanthine bases (xanthine, guanine, etc.). |
| 32 | Characteristic odour. Solidifies at 4°, melting again at about 14°. Gives an acid reaction only in alcoholic solution. To 1 ml. of oleic acid and 3 ml. of saturated aqueous solution of sodium nitrite add 1 ml. of sulphuric acid; on standing for a few hours the solid stereoisomeride elaidic acid is formed. |
| 33 | A neutralised solution gives a ppt. with calcium chloride insoluble in acetic acid but soluble in hydrochloric acid. The ppt. mixed with dilute sulphuric acid decolorises potassium permanganate on warming. |
| 34 | Commercial acid has low acid value—not less than 120. Yields ricinelaidic acid with H_2SO_4 and saturated sodium nitrite solution. |
| 35 | Aqueous or alcoholic solutions give a purple colour with 1 drop of ferric chloride solution, just visible with 0.0025 mg. in 1 ml. |
| 36 | Acid value 200 to 210. |
| 37 | Forms fluorescein dyes when heated with resorcin and sulphuric acid. |
| 38 | Neutral solution of salt gives brown ppt. with ferric chloride; when ppt. is washed, dissolved in ammonia and the solution filtered, the filtrate gives a ppt. with barium chloride and equal volume of alcohol. |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|------------------------|--|----------------------|----------------------------------|-------------------------------------|---------------------------------|---------------------------------------|
| 39 | Acid Tannic | C ₇ H ₆ O | — | — | — | 1·5 | 1 |
| 40 | Acid Tartaric | C ₄ H ₄ O | 162-169 | — | — | 0·8 | 2·5 |
| 41 | Acid Tri-chloroacetic | C ₂ H ₂ O ₂ Cl | 55 | 195 | — | About 0·11 | Readily |
| 42 | Acid Valerianic | C ₅ H ₈ O | — | — | 0·93 | 30 | Miscible |
| 43 | Aconitine | C ₃₄ H ₄₈ O ₆ N | 196-200 (Decomp.) | — | — | Very slight | 40 |
| 44 | Acriflavine | C ₁₂ H ₈ N ₂ Cl | — | — | — | About 3 | Soluble |
| 45 | Adrenaline | C ₉ H ₉ O ₃ N | 205-212 | — | — | Very slight | Very slight |
| 46 | Æsculin | C ₁₆ H ₁₂ O | — | — | — | Slight | Slight |
| 47 | Albumin Tannate | C ₁₂ H ₁₀ O ₂ N | — | — | — | Slight | Slight |
| 48 | Alcohol Cetyl | C ₁₈ H ₃₈ O | 48-52 | — | — | Insoluble | Soluble in hot alcohol |
| 49 | Alcohol Isopropyl | C ₃ H ₈ O | — | 80·5-81·5 (82·4 if anhydrous) | 0·810-0·812 (0·788 if anhydrous) | Miscible | Miscible |
| 50 | Alcohol Methyl | C ₂ H ₆ O | — | 66 | About 0·798 | Miscible | Miscible |
| 51 | Alcohol Tri-bromoethyl | C ₂ H ₃ OBr | 79-81 | — | — | 35 at 25° | Soluble |

| No. | SPECIAL TESTS |
|-----|---|
| 39 | Gives ppt. with gelatin, lead nitrate solution (with 0.0001 g.), bismuth nitrate solution (with 0.0001 g.), and ammoniacal copper solution (distinctions from gallic acid). 0.0001 g. gives blue with ferric chloride. Is hydrolysed into gallic acid and dextrose by boiling with dilute sulphuric acid. Gives brown with sodium nitrite solution. |
| 40 | To a neutral solution add silver nitrate; white silver tartrate is pptd. On just dissolving in dilute ammonia and warming, a silver mirror is obtained if test-tube is clean. To aqueous solution add 1 drop ferrous sulphate, 3 drops hydrogen peroxide and excess sodium hydroxide; a deep violet colour is produced. |
| 41 | When boiled with water and sodium hydroxide solution, chloroform is produced. |
| 42 | Has a characteristic, unpleasant odour. Fused calcium chloride separates the acid from its aqueous solution. Zn and Ag salts are insoluble. |
| 43 | 0.001 g. warmed with 4 drops sulphuric acid (sp. gr. 1.75) and a crystal of resorcinol added, the liquid becomes reddish-violet in about 20 minutes. 0.000001 g. produces tingling and numbing on the tongue. 0.0001 g. with dilute acetic acid gives red ppt. with potassium permanganate, not changed with bromine water. |
| 44 | Dilute solution is yellow, strong solutions red with deep green fluorescence; gives hydrogen chloride on heating with sulphuric acid (distinction from proflavine). Bulky yellow ppt. with 10% solution of sodium salicylate. |
| 45 | Sodium nitrite alone gives red colour. Reduces silver nitrate solution. Faintly acid solution with 0.25% ferric chloride solution gives emerald-green colour; on gradual addition of sodium bicarbonate solution colour changes through blue to red. |
| 46 | Yields dextrose and æsculetin on hydrolysis and then reduces Fehling's. Gives blood-red colour when treated with nitric acid and then excess of ammonia. Gives blue fluorescence in alkaline solution. Treated with sulphuric acid and then solution of sodium hypochlorite it gives a violet colour. |
| 47 | Decomposed by aqueous alkali hydroxides and carbonates. |
| 48 | Crystalline and greasy to the touch. Iodine value, not more than 20 for commercial alcohol. |
| 49 | Heated with sulphuric acid it gives propylene (characteristic odour). Oxidised with 4 vols. of 10% potassium dichromate and 1 vol. of sulphuric acid, acetone is evolved. |
| 50 | Oxidised by hot copper wire, coated with oxide, to formaldehyde. |
| 51 | Unstable; after hydrolysis with warm NaOH solution, gives reactions for bromides. Usually met with in amylene hydrate solution as avertin. |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|--|------------------------|--------------|-------------|-----------------|---------------------------------|--|
| 52 | Allantoin | $C_4H_6O_4N_2$ | About 235 | — | — | 130 | Very slight |
| 53 | Allobarbitone | $C_8H_{10}O_4N_2$ | 171-172 | — | — | Slight | Slight in cold, very soluble in hot |
| 54 | Aloin | $C_{12}H_{18}O_5$ | 145 | — | — | 140 | 20 |
| 55 | Amethocaine Hydrochloride (Butethanol) | $C_{11}H_{15}O_3N_2Cl$ | 143-150 | — | — | 7 | Soluble |
| 56 | Amidopyrine | $C_8H_7O_3N_3$ | 107-109 | — | — | 18 | About 2 |
| 57 | Ammonium Mandelate | $C_8H_9O_3N$ | — | — | — | Very soluble | Soluble |
| 58 | Amphetamine | $C_9H_{11}N$ | — | 200- 203 | 0.931 | Slightly soluble | Soluble |
| 59 | Amphetamine Sulphate | $C_9H_{11}O_4N_2S$ | About 300 | — | — | Soluble | Slightly Soluble |
| 60 | Amydricaine Hydrochloride | $C_{12}H_{15}O_3N_2Cl$ | About 169 | — | — | 1 | 4 |
| 61 | Amyl Nitrite | $C_5H_{11}O_2N$ | — | 90-100 | 0.874- 0.884 | Insoluble | Miscible |
| 62 | Amyl Valerianate | $C_{11}H_{20}O_3$ | — | 204 | 0.881 | Very slight | Miscible |
| 63 | Amylene Hydrate | C_4H_8O | — | 97-103 | 0.812- 0.815 | 8 | Miscible |
| 64 | Amylethyl- barbitone Soluble (Amytal Sodium) | $C_{12}H_{15}O_4N_2Na$ | — | — | — | Soluble | Freely |

| No. | SPECIAL TESTS |
|-----|--|
| 52 | Conc. furfural solution to which a little hydrochloric acid is added gives a violet colour with an aqueous solution. Mercuric nitrate (not chloride) gives a ppt. as with urea. |
| 53 | 0.1 g. in 1 ml. sulphuric acid gives yellow solution changing slowly to dark red. 0.001 g. heated with sodium hydroxide solution and acidified with sulphuric acid gives carbon dioxide with acetic odour. Saturated aqueous solution gives white ppt. with Millon's reagent, soluble in excess. |
| 54 | Dissolves in ammonia and caustic alkalis, the yellow solution rapidly turning red with green fluorescence. Ferric chloride added to alcoholic solution gives brown-green colour. One drop of copper sulphate solution added to 0.00005 g. in 5 ml. water gives yellow colour changed to red by 0.5 ml. saturated sodium chloride and to violet by 1 ml. 90% alcohol. |
| 55 | Surface anæsthetic, formerly known as Decicain. |
| 56 | Silver nitrate gives violet colour followed by ppt. of metallic silver. Ferric chloride gives bluish-violet. Sodium nitrite and hydrochloric acid give violet. |
| 57 | Very hygroscopic. Gives ammonia and benzaldehyde when heated. |
| 58 | Volatilises readily at ordinary temperatures. The 10% solution in NaOH with benzoyl chloride gives benzoyl derivative which melts at 134°-135°. |
| 59 | Gives reaction of sulphates and can be identified by m.p. of the benzoyl derivative of base (<i>see under</i> Amphetamine). |
| 60 | Behaves similarly to cocaine, <i>q.v.</i> Can be distinguished by fact that 4% solution does not precipitate with platinic chloride in presence of hydrochloric acid. |
| 61 | Characteristic odour—produces flushing of face on inhalation. |
| 62 | Yields amyl alcohol and valeric acid on hydrolysis. |
| 63 | Has a pungent taste, and an odour resembling camphor and peppermint. It solidifies to crystals on cooling to a low temperature. Oxidation with chromic acid gives acetone and acetic acid. |
| 64 | Isomeric with Pentobarbitone Soluble; contains isoamyl groups instead of methylbutyl. Gives general reactions of malonylureas; free acid melts 153°-155°. |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|--|-------------------------|---|--------------|-----------------|---------------------------------|---------------------------------------|
| 65 | Amylocaine Hydro- chloride | $C_9H_{10}O_2N, Cl$ | 177-179 | — | — | 2 | 3 |
| 66 | Andro- sterone | $C_{27}H_{48}O$ | About 180° | — | — | Insoluble | Soluble |
| 67 | Aneurine Hydro- chloride | $C_8H_{10}O_2N, S, Cl$ | 245-250 after drying at 105° | — | — | Soluble | Slightly Soluble |
| 68 | Aniline | C_6H_5N | — | About 183 | About 1.027 | 37 | Miscible |
| 69 | Anthrarobin | $C_{15}H_{10}O$ | — | — | — | Very slight | 80 |
| 70 | Apiol | $C_{15}H_{20}O$ | — | — | 1.055- 1.091 | Insoluble | Partly soluble |
| 71 | Apocodeine Hydro- chloride | $C_{18}H_{21}O_2N, Cl$ | Partly at 90, decom- poses over 200 | — | — | 1 | 1 |
| 72 | Apomorphine Hydro- chloride | $C_{17}H_{19}O_2N, Cl$ | — | — | — | 60 | 51 |
| 73 | Arbutin | $C_8H_{10}O_4$ | About 168 | — | — | 10 | 13 |
| 74 | Arecoline Hydro- bromide | $C_8H_{11}O_2N, Br$ | About 167-170 | — | — | Readily | Readily |
| 75 | Arsphena- mine | $C_8H_{10}O_2N, Cl, As$ | — | — | — | Readily | Slight |

| No. | SPECIAL TESTS |
|-----|---|
| 65 | Distinguished from cocaine by giving no ppt. with potassium permanganate but decolorising it slowly. Aqueous solution gives ppt. on addition of iodine solution (distinction from orthocaine) and with Mayer's reagent (distinction from orthocaine and benzocaine). |
| 66 | An androgenic hormone in male urine; usually met with in oily solution in ampoules. |
| 67 | The picrate melts at 206° to 208°. 0.01 g. in 10 ml. of 2N NaOH and 10 ml. of potassium ferricyanide solution, shaken for 2 minutes with butyl alcohol, gives intense blue fluorescence in separated alcoholic layer (thiocrome reaction). Trace in 0.3 ml. of mixture of 7 vols. of N/1000 HCl and 3 vols. of alcohol, with 1 drop of formaldehyde solution and a solution consisting of 1.25 ml. of mixture of equal vols. of N/1 NaOH and 5.76% sodium bicarbonate solution and 0.5 ml. of diazobenzenesulphonic acid solution, gives pink colour on standing which forms pink layer when shaken with butyl alcohol and allowed to separate. |
| 68 | To neutral or slightly alkaline solution add sodium hypochlorite or chlorinated lime solution—purple-violet, even in 1 in 26,000, changing to dirty red. Avoid excess of reagent. When this change has occurred add ammoniacal phenol solution; return of blue colour even in 1 in 66,000. Aqueous chromic acid solution, according to concentration, gives green, blue or almost black. |
| 69 | Easily soluble in caustic alkalis and ammonia, giving yellowish solution gradually changing to green or blue owing to formation of alizarin. |
| 70 | A green liquid with characteristic odour and taste. |
| 71 | Occurs usually as a yellowish or greenish-grey hygroscopic powder. Gives a characteristic blood-red colour with nitric acid. |
| 72 | On adding ammonium persulphate and sodium bicarbonate to aqueous solution and shaking with chloroform the latter becomes a red or violet. Detects 1 in 100,000. Acquires a green tint when exposed to light and air, and dissolves in nitric acid with purple colour. Dilute aqueous solution with sodium bicarbonate solution gives ppt. which is white at first, becoming green. Ppt. dissolves in alcohol (green), chloroform (blue) and ether (purple). |
| 73 | Hydrolysed by dilute sulphuric acid into dextrose and hydroquinone. Diazo Test—yellow with sodium nitrite solution and hydrochloric acid turning red with sodium hydroxide. |
| 74 | Aqueous solution gives with mercuric chloride solution a white ppt. soluble in excess, the solution depositing colourless crystals on standing a few hours. Sulphuric and selenious acids give a bright yellow colour (distinction from pelletierine). |
| 75 | 1 ml. of 1% solution with 1 drop of 5% sodium nitrite gives orange colour. 0.5 ml. of resulting solution with 1 ml. 2% β -naphthol in 2N sodium hydroxide gives a clear wine-red solution. |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|---------------------------------|---------------------------|------------------------------------|-------------------------------|-----------------|---------------------------------|---------------------------------------|
| 76 | Asparagin | $C_4H_8O_3N$ | — | — | — | 50 | Insoluble |
| 77 | Atropine | $C_{17}H_{27}O_3N$ | 114–116 | — | — | 300 | 3 |
| 78 | Atropine Methyl- nitrate | $C_{17}H_{27}O_3N$ | De- comp. | — | — | Soluble | Soluble |
| 79 | Atropine Sulphate | $C_{17}H_{27}O_3N$, S | 195–196 (when dry) | — | — | 0.5 | 3 |
| 80 | Barbitone | $C_4H_4O_3N_2$ | 189–192 | — | — | 170 | 8.5 |
| 81 | Barbitone Soluble | $C_4H_4O_3N_2$, Na | — | — | — | 5 | Slight |
| 82 | Beberine Sulphate | $C_{17}H_{27}O_3N$, S | — | — | — | 1 | Slight |
| 83 | Benzamine Hydro- chloride | $C_7H_9O_2N$, Cl | About 268 (de- comp.) | — | — | 30 | 35 |
| 84 | Benzamine Lactate | $C_7H_9O_2N$ | 152–156 | — | — | 5 | 8 |
| 85 | Benzene | C_6H_6 | About 0°; re- melts at 4° | 79–82 (80 when pure) | 0.880– 0.887 | Insoluble | Miscible |
| 86 | Benzocaine | $C_9H_9O_2N$ | 90–91 | — | — | 2500 | 8 |
| 87 | Benzyl Benzoate | $C_{15}H_{13}O_2$ | 20 | About 323 | 1.121– 1.125 | Insoluble | Miscible |

| No. | SPECIAL TESTS |
|-----|---|
| 76 | In alkaline solution is laevorotatory; in acid, dextro. Copper hydroxide is dissolved on boiling, forming a blue solution, depositing on cooling asparagin-copper ($C_4H_7O_2N_2$) ₂ Cu. Insoluble in ether. |
| 77 | 0.001 g. warmed with 2 ml. mercuric chloride in 50% alcohol causes deposition of mercuric oxide (with some mercurous oxide). Dilates the pupil even 1 in 130,000. 0.01 mg. responds to VITALI'S REACTION: Evaporate a trace of atropine (or a salt) in a porcelain dish with a few drops of fuming nitric acid; a yellow residue is produced which on moistening with alcoholic potassium hydroxide (1 in 10) produces a violet colour. Strychnine does the same on applying 4% potash, but the colour is evanescent. Veratrine gives red-violet or orange-red. |
| 78 | Yields reactions of atropine and on saponification with alc. KOH produces potassium nitrate. |
| 79 | Gives aurichloride, m.p. 137° to 139°. |
| 80 | A saturated solution acidified with nitric acid gives ppt. with Millon's reagent, soluble in excess. When heated with sodium hydroxide (solid or conc. solution), ammonia is evolved. |
| 81 | Aqueous solution gives ppt. of barbitone on acidifying. |
| 82 | Occurs in brown translucent scales and contains about 30% of beberine with other alkaloids. |
| 83 | Benzamine lactate and benzamine hydrochloride—0.1 g. in 1 ml. sulphuric acid maintained at 100° for 5 minutes and cautiously diluted with 2 ml. water gives aromatic odour and on cooling deposits crystals of benzoic acid. |
| 84 | 0.05 g. with 0.25 g. calomel does not blacken when moistened with water (distinction from cocaine hydrochloride). 5 ml. of 1% solution gives no permanent ppt. with 5 ml. mercuric chloride solution or with 5 ml. potassium iodide solution (distinction from cocaine and alpha-caine respectively). 4% solution of the hydrochloride gives a slight golden-brown ppt. with platinic chloride, dissolving in hydrochloric acid and separating again in crystals on standing. |
| 85 | To distinguish from petroleum benzene note solubility in alcohol; benzene is soluble with half volume of alcohol 90%, but petroleum benzene requires 5 to 6 volumes (using petrol, more). Warm 1 ml. with 5 to 10 ml. of mixture of nitric and sulphuric acids (2 : 1); benzene gives red vapour and yellow nitro-compounds; on diluting with water an odour of benzaldehyde is evolved. Petroleum benzene is practically unaffected. |
| 86 | 1 ml. 1% aqueous solution with trace of hydrochloric acid and 1 to 2 drops of sodium nitrite solution followed by 1 to 2 drops of β -naphthol in sodium hydroxide solution gives a deep red colour and, on standing, scarlet ppt. |
| 87 | Decomposes into benzyl alcohol and sodium benzoate on boiling with sodium hydroxide. |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|---|------------------|-------------|-------------|---------------|---------------------------------|---------------------------------------|
| 88 | Benzyl Hydroxy- benzoate | C,H,O | 110 | — | — | About 10,000 | Soluble |
| 89 | Benzyl Succinate | C,H,O | 45-47 | — | — | Almost insoluble | Readily |
| 90 | Benzyl- morphine Hydro- chloride | C,H,O,N, Cl | — | — | — | 133 | 218 |
| 91 | Berberine Sulphate | C,H,O,N, S | — | — | — | 150 | Soluble |
| 92 | Betaine Hydro- chloride | C,H,O,N, Cl | — | — | — | 2 | About 20 |
| 93 | Beta- naphthol | C,H,O | 120-122 | — | — | 1000 | 2 |
| 94 | Bismuth Citrate | C,H,O,Bi | — | — | — | Insoluble | Insoluble |
| 95 | Bismuth Salicylate | C,H,O,Bi | — | — | — | Insoluble | Insoluble |
| 96 | Bismuth Sodium Tartrate | C,H,O,Na, Bi | — | — | — | Soluble | — |
| 97 | Bismuth Sub- gallate | C,H,O,Bi | — | — | — | Insoluble | Insoluble |
| 98 | Bismuth Tribromo- phenate | C,H,O,Br, Bi | — | — | — | Insoluble | Insoluble |
| 99 | Bordeaux B | C,H,O,N, S,Na | — | — | — | Soluble | Soluble |
| 100 | Bromal Hydrate | C,H,O,Br | 54 | — | — | 2.5 | 0.5 |
| 101 | Bromoform | C,H,Br | — | 148- 155 | About 2.63 | 800 | Miscible |
| 102 | Bromoiso- valerianyl- urea | C,H,O,N, Br | 145 | — | — | Slight | 10 |

| No. | SPECIAL TESTS |
|-----|---|
| 88 | Hydrolysis with alkali yields benzyl alcohol and <i>p</i> -hydroxybenzoic acid. A trace boiled with water gives reddish-violet solution with 1 drop of ferric chloride changing to brown on adding further 2 drops mercuric nitrate to boiling sol. gives ppt. and red supernatant liquid. |
| 89 | Hydrolysed to benzyl alcohol and sodium succinate on boiling with sodium hydroxide. |
| 90 | With conc. sulphuric acid gives yellow colour which on warming gradually darkens through brownish-red to dark red; conc. nitric acid gives pale yellow colour; ammonium molybdate and conc. sulphuric acid gives a violet, changing through brown to a permanent blue. |
| 91 | To 5 ml. of 1% aqueous solution add 2 drops sodium hydroxide solution; the liquid acquires an orange red colour but gives no ppt. On adding 4 drops acetone, it becomes turbid and gives yellow ppt. on standing. |
| 92 | Gold salt melts at 224°. Has a strongly acid reaction and taste. Fusion with potassium hydroxide gives trimethylamine. |
| 93 | Gives bluish fluorescence on adding 1 ml. solution of ammonia to 10 ml. saturated aqueous solution. |
| 94 | Soluble in ammonia and alkali citrates. Gives citrate and bismuth reactions. |
| 95 | Identify bismuth and salicylic acid—the latter by shaking powder with ether and dilute sulphuric acid; on shaking ethereal layer with water containing trace of ferric chloride, a violet colour is produced. |
| 96 | Gives reactions of bismuth, sodium and after removal of bismuth, of tartrate. |
| 97 | Soluble in hot mineral acids and in alkali hydroxide giving a yellow solution rapidly changing to deep red. When suspended in water and the bismuth removed by hydrogen sulphide, the filtrate gives a bluish black colour with ferric chloride. |
| 98 | Boiled with sodium hydroxide solution and the liquid acidified, a ppt. of tribromophenol is produced which when washed and dried has m.p. 90° to 95°. |
| 99 | A 1 in 1000 solution acquires a brownish-red coloration with excess of sodium hydroxide solution and a blue coloration with concentrated sulphuric acid. |
| 100 | Decomposes at 100° to 110° into bromal and water. |
| 101 | Has odour resembling chloroform, from which it is easily distinguished by sp. gr. and b.p. |
| 102 | Odour like bromal on heating. Dissolves in caustic alkali solution and is pptd. by acids. After heating with sodium hydroxide and acidifying the residue with sulphuric acid, carbon dioxide is liberated with valerianic odour. Millon's reagent gives ppt. (<i>cf.</i> barbitone). Nessler's reagent with saturated solution gives only slight colour and ppt. (<i>cf.</i> carbromal), but after fusing with potassium hydroxide and dissolving in water there is the usual effect. |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90 % (1 in -) |
|-----|--------------------------------------|------------------|--|-------------|------------|---------------------------------|--|
| 103 | Brucine | C,H,O,N | 178 (when anhyd.) | — | — | Insoluble | Soluble |
| 104 | Butylchloral Hydrate | C,H,O,Cl | About 78 | — | — | 40 | 0.6 |
| 105 | Butylethyl- barbituric Acid | C,H,O,N | 124-127 | — | — | 300 | Soluble |
| 106 | Caffeine | C,H,O,N | 235-237 (dried at 100°) | — | — | 80 | 40 |
| 107 | Caffeine Citrate | C,H,O,N | About 160 | — | — | 32 | 25 |
| 108 | Caffeine and Sodium Benzoate | C,H,O,N, Na | — | — | — | 4 | Slight |
| 109 | Caffeine and Sodium Iodide | C,H,O,N, Na,I | — | — | — | Soluble | Partly soluble |
| 110 | Caffeine and Sodium Salicylate | C,H,O,N, Na | — | — | — | 1 | 28 |
| 111 | Calciferol | C,H,O | 115-119 (in eva- cuated tube) | — | — | Insoluble | Readily |
| 112 | Calcium Acetyl- salicylate | C,H,O,Ca | — | — | — | 6 | 800 |
| 113 | Calcium Gluconate | C,H,O,Ca | — | — | — | 30 | Insoluble |
| 114 | Calcium Glycero- phosphate | C,H,O,P, Ca | — | — | — | About 50 | Insoluble |
| 115 | Calcium Lactate | C,H,O,Ca | — | — | — | 18.5 | Slight |
| 116 | Calcium Levulinate | C,H,O,Ca | — | — | — | 2.5 | Insoluble |

| No. | SPECIAL TESTS |
|-----|---|
| 103 | Yields normal reactions of alkaloids and red colour with conc. HNO_3 . No violet colour with sulphuric acid and potassium dichromate (distinction from strychnine). |
| 104 | Nitric acid converts it into trichlorobutyric acid, m.p. 44° . |
| 105 | Dissolves in aqueous alkali hydroxides; heated with conc. NaOH , ammonia is evolved. |
| 106 | On evaporating 0.0001 g. to dryness with bromine water a reddish-brown spot is obtained, which turns violet-red with ammonia vapour (murexide reaction). Aqueous solution with N/10 iodine gives no ppt. unless acidified with hydrochloric acid. |
| 107 | The aqueous solution is acid owing to hydrolysis. All the citric acid present can be titrated with caustic soda using phenolphthalein as indicator. Murexide test as above. |
| 108 | Gives sublimate of caffeine when heated in dry tube. Gives reactions of caffeine and sodium benzoate. Caffeine can be extracted with chloroform from alkaline solution. |
| 109 | Gives reactions of caffeine and sodium iodide. Caffeine sublimes on heating. |
| 110 | Gives reactions of caffeine and sodium salicylate. Caffeine can be estimated by extraction with chloroform from alkaline solution. |
| 111 | The 3:5-dinitrobenzoate has m.p. 147° to 149° . |
| 112 | Hydrochloric acid ppts. aspirin; filtrate gives reactions for calcium. After hydrolysis, gives reactions for salicylic acid. |
| 113 | 2% aqueous solution gives a yellow colour with 1 drop of ferric chloride solution. The aqueous solution gives when heated for 30 minutes on a water-bath with acetic acid and phenylhydrazine and cooled, white crystals of gluconic acid phenylhydrazide which, when recrystallised from hot aqueous solution and filtered through decolorising charcoal, have a m.p. of 200° to 202° (decomp.). |
| 114 | Identify calcium and phosphate after hydrolysis with acid. |
| 115 | Acidify a 1 in 20 solution with sulphuric acid, add potassium permanganate and heat—the odour of acetaldehyde is developed. |
| 116 | Heating with acetic acid and phenylhydrazine on water-bath gives characteristic phenylhydrazide. Decolorises KMnO_4 and gives reactions for calcium. |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in—) | SOL. IN ALCOHOL 90% (1 in—) |
|-----|------------------------------|-----------------------------|-------------------------|---------------|-----------------|-------------------------------------|---|
| 117 | Calcium Saccharate | $C_7H_{10}O_5Ca$ | — | — | — | 10 | Insoluble |
| 118 | Calcium Sodium Lactate | $C_3H_5O_3Ca$ Na | — | — | — | About 15 | 10 in boil- ing alcohol |
| 119 | Camphor | $C_{15}H_{26}O$ | 174–177 | — | — | 700 | 1 |
| 120 | Camphor Mono- bromide | $C_{15}H_{25}OBr$ | 74–76 | — | — | Insoluble | 12 |
| 121 | Cantharidin | $C_{12}H_8O_4$ | 216–218 | — | — | 1100 | Slight |
| 122 | Carbachol | $C_7H_{10}O_2N$, Cl | 210–212 | — | — | Very soluble | Slightly soluble |
| 123 | Carbon Tetra- chloride | C_2Cl_4 | — | 76·5– 77·5 | 1·603– 1·606 | Insoluble | Miscible (with de- hydrated alcohol) |
| 124 | Carbromal | $C_7H_5O_2N$, Br | 116–118 | — | — | 3000 (more soluble in hot) | 18 |
| 125 | Chiniofon | $C_7H_5O_2N$, S, I | De- comp. at 275° | — | — | 25 | Insoluble |
| 126 | Chloral Formamide | $C_2H_3O_2N$, Cl | 114–115 | — | — | 21 | 2 |
| 127 | Chloral Hydrate | $C_2H_3O_2Cl$ | 50–58 | — | — | 0·25 | 0·2 |
| 128 | Chloramine | $C_2H_3O_2N$, Cl, S, Na | De- comp. | — | — | 7 | 12 |

| No. | SPECIAL TESTS |
|-----|--|
| 117 | To a suspension of a small quantity in 10 ml. of water add 5 ml. of hydrochloric acid and 0.1 g. of resorcinol in 5 ml. of water; shake and heat in a boiling water-bath for 5 minutes. A pink to red colour is produced. The test can be used to detect the compound in cream, using 10 ml. |
| 118 | Gives reactions of calcium and sodium. On warming with potassium permanganate gives acetaldehyde. |
| 119 | Characteristic odour. |
| 120 | Alcoholic potassium hydroxide has no action, but silver oxide in presence of chloroform decomposes it. Heated with 4 times its quantity of nitric acid on sand-bath forms camphoric acid and bromonitro-camphor—rhombic prisms almost insoluble in alcohol, m.p. 105°. |
| 121 | Boiled with soda and potash forms cantharidates. An exceedingly minute quantity of cantharidin will produce a blister. |
| 122 | Hydrosopic with fishy odour. Solution gives reaction for chlorides. Heated with NaOH sol. ammonia is evolved and then trimethylamine. Auric chloride cpd. after drying at 100°, melts at 183° to 184°. Cream-coloured ppt. with potassio-mercuric iodide. |
| 123 | The vapour of volatile halogen compounds imparts a green tinge to a colourless flame. |
| 124 | Sulphuric acid gives hydrogen bromide on warming. Sulphuric acid with manganese dioxide gives bromine. Heated with dilute sodium hydroxide solution, ammonia is evolved and sodium bromide formed. Does not give ppt. with Millon's reagent after acidifying (<i>cf.</i> barbitone). Saturated aqueous solution gives yellow with Nessler's reagent and then orange ppt. (Allobarbitone, barbitone and propional do not react unless fused with potassium hydroxide previously; bromoisovalerylurea and phenobarbitone give only a faint colour.) |
| 125 | Light yellow pdr. Saturated solution in H ₂ O with HCl changes colour from orange to yellow and produces yel. xtal. ppt. 1% solution gives olive-green with ferric chloride and white ppt. with copper sulphate. Nitrous acid liberates iodine. Contains nearly 30% iodine and about 20% NaHCO ₃ . |
| 126 | Water slightly warm decomposes it. Caustic alkalis decompose it into chloroform, ammonia and alkali formate. Dilute acids have no action. |
| 127 | Mixed with sodium hydroxide solution chloroform is produced. Compounds containing the group CX ₃ (X=Cl, Br, or I) on heating with 20% sodium hydroxide solution and pyridine give a red colour which passes into pyridine layer. Reaction detects less than 0.005 mg. of chloral hydrate or chloroform. |
| 128 | On ignition decomposition occurs suddenly, sodium sulphate being produced. Warmed with sodium hydroxide and aniline gives phenyl isocyanide odour. Liberates iodine from potassium iodide solution. Does not liberate bromine from neutral sodium bromide solution and gives white ppt. with mercuric chloride solution (distinction from dichloramine). |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|--------------------------|--|----------------------------------|-------------------------------------|-------------|---------------------------------|---------------------------------------|
| 129 | Chlorbutol | C ₇ H ₇ O ₂ Cl | Not below 78 (96 when anhydrous) | 167 | — | 125 | 1 |
| 130 | Chlorocresol | C ₇ H ₇ O ₂ Cl | 64-66 | — | — | 250 | Soluble |
| 131 | Chloroform | C ₂ H ₂ Cl | — | Port'n below 60; remainder at 60-62 | 1.485-1.490 | 200 | Miscible |
| 132 | Chloroxylenol | C ₇ H ₇ O ₂ Cl | 114-115.5 | — | — | 3000 | Soluble |
| 133 | Cholesterol | C ₂₇ H ₄₈ O | About 145 | — | — | Slightly | Soluble (hot) |
| 134 | Chrysarobin | C ₁₂ H ₈ O | 155-165 | — | — | Almost insoluble | Slight |
| 135 | Cinchonidine | C ₂₁ H ₂₂ O ₄ N | 202 | — | — | Very slight | 16 |
| 136 | Cinchonidine Sulphate | C ₂₁ H ₂₂ O ₄ N ₂ S | About 207 (when anhyd.) | — | — | 100 | 60 |
| 137 | Cinchonine | C ₂₁ H ₂₂ O ₄ N | 255 | — | — | Very slight | 175 |
| 138 | Cinchonine Hydrochloride | C ₂₁ H ₂₂ O ₄ N ₂ Cl | 217-218 (when anhyd.) | — | — | 22 | 1 |
| 139 | Cinchophen | C ₁₂ H ₈ O ₂ N | 214-217 | — | — | Insoluble | 120 |
| 140 | Cinnamic Aldehyde | C ₉ H ₈ O | — | 252 (slight decomp.) | 1.054-1.057 | Slight | Miscible |

| No. | SPECIAL TESTS |
|-----|---|
| 129 | Gives phenyl isocyanide odour on warming with aniline and sodium hydroxide solution. Iodoform is produced on shaking with iodine and dilute sodium hydroxide solution. |
| 130 | Violet with ferric chloride; gives reactions for chlorides after fusion with sodium carbonate. |
| 131 | Gives phenyl isocyanide odour on warming with sodium hydroxide and a trace of aniline. |
| 132 | After heating with anhydrous sodium carbonate gives reactions for chlorides. No violet colour with FeCl_3 (distinction from chlorocresol). |
| 133 | The white crystalline compound obtained from wool-fat by saponification with alc. KOH. |
| 134 | Mix a trace with 1 drop of fuming nitric acid and add 1 drop of dilute ammonia solution; an evanescent violet coloration is produced. When shaken with dilute ammonia solution mixed with twice its volume of water and the solution filtered, the filtrate slowly acquires a pink colour. |
| 135 | Gives neither thalleioquin test nor the potassium ferricyanide modification (<i>cf.</i> Cinchonine). Soluble in large amounts of ether. Sodium potassium tartrate added to neutral solution of a salt gives white precipitate. |
| 136 | Gives reactions of cinchonidine and sulphate. |
| 137 | Only slightly soluble in ether (1 in 370). Few characteristic reactions. Not pptd. by sodium bicarbonate in presence of tartaric acid (quinine and cinchonidine are). Does not give thalleioquin test nor red colour with potassium ferricyanide and ammonia on addition of these to acetic acid solution after treating with bromine (distinction from quinine and quinidine). Not rendered fluorescent by very dilute sulphuric acid. |
| 138 | Gives reactions of cinchonine and hydrochloride. |
| 139 | Saturated solution in hot hydrochloric acid gives brown crystalline ppt. with platonic chloride solution. Ammoniacal solution evaporated to small bulk and diluted with water gives a white ppt. with silver nitrate, a yellow ppt. with lead acetate and a green ppt. with copper sulphate. |
| 140 | Oxidised by potassium permanganate and acid into benzaldehyde and benzoic acid. |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|-----------------------|----------------|-------------------------|------------------------------------|----------------------|---------------------------------|---------------------------------------|
| 141 | Cocaine | C,H,O,N | 97-98 | — | — | Very slight | 10 |
| 142 | Cocaine Hydrochloride | C,H,O,N, Cl | Not below 197 | — | — | 0.5 | 3 |
| 143 | Codeine | C,H,O,N | 155-156 | — | — | 120 | 20 |
| 144 | Codeine Phosphate | C,H,O,N, P | — | — | — | 3.5 | 350 |
| 145 | Colchicine | C,H,O,N | About 145 (when anhyd.) | — | — | 1 | 1 |
| 146 | Colchicine Salicylate | C,H,O,N | 55-60 | — | — | Slight | 1 |
| 147 | Collargol | C,H,O,N, Ag | — | — | — | 25 | Insoluble |
| 148 | Coniine Hydrobromide | C,H,N, Br | About 212 | — | — | 2 | 3 |
| 149 | Cotarnine Chloride | C,H,O,N, Cl | About 125 | — | — | Less than 0.5 | 3 |
| 150 | Cotarnine Phthalate | C,H,O,N | 113 | — | — | 0.5 | 5 |
| 151 | Coumarin | C,H,O | 68-70 | — | — | 500 | 7 |
| 152 | Creosote | C,H,O | — | 200-230 | Not lower than 1.070 | 150 | Miscible |
| 153 | Cresol | C,H,O | — | Mainly distills betw'n 195 and 205 | 1.035-1.050 | 50 | Miscible |
| 154 | Cyclohexane | C,H | — | 81 | 0.779 | Not miscible | Not miscible |

| No. | SPECIAL TESTS |
|-----|---|
| 141 | <i>See Cocaine Hydrochloride.</i> |
| 142 | Addition of 1 to 2 drops chromic acid solution to 1% cocaine gives a yellow ppt. re-dissolved on shaking; with more chromic acid ppt. is permanent. |
| 143 | Does not reduce iodic acid (distinction from morphine). No blue colour with ferricyanide and ferric chloride (distinction from morphine). 0.001 g. warmed with 1 ml. sulphuric acid and 2 drops ferric chloride solution gives deep blue colour. Greenish-blue with Froehde's reagent. |
| 144 | Gives reactions of codeine and phosphate. |
| 145 | Only slightly soluble in ether or benzene. Practically insoluble in light petroleum. Sulphuric acid with a trace of nitric acid added gives yellowish-green, changing through blue-violet and wine-red to yellow. Chlorine water added to the aqueous solution gives yellow ppt. soluble in ammonia forming an orange coloured solution. Sodium nitrite and hydrochloric acid give dirty brown. |
| 146 | Gives reactions of colchicine and salicylic acid. |
| 147 | A substance having a black or metallic appearance and containing about 90% silver. |
| 148 | With conc. sulphuric acid gives a blood-red colour turning green. This liquid alkaloid is distinguished from nicotine by giving no ppt. with platinic chloride, and a deep red colour with alkaline solution of phenolphthalein. |
| 149 | 0.3 g. dissolved in 5 to 15 ml. water and N/10 iodine solution added gives brown ppt. which dried over sulphuric acid melts at 142° to 144°. Cotarnine dissolves in conc. nitric acid with formation of a red solution and oxalic acid. |
| 150 | Decompose salt and identify the alkaloid and phthalic acid. |
| 151 | Has a characteristic fragrant odour and sublimes at 100°. Dissolves slowly in hot sodium hydroxide solution with a slight green colour, excess of acid reprecipitating coumarin. Saturated aqueous solution with iodine solution gives a brown flocculent ppt. which coagulates to a dark green mass on shaking. |
| 152 | 1 drop of ferric chloride solution added to saturated aqueous solution gives a transient violet-blue colour; with more ferric chloride the solution becomes cloudy, the colour changing through greyish-green to brown. |
| 153 | Ferric chloride solution added to a dilute aqueous solution gives a transient bluish colour. Bromine water gives a pale yellow flocculent ppt. |
| 154 | Reactions characteristic of saturated aliphatic hydrocarbon. |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|------------------------------|------------------|---------------------|-------------|-------------------|---------------------------------|---------------------------------------|
| 155 | Cyclopropane | C,H | — | -34.5 | 1.46 (density) | 2.7 | Soluble |
| 156 | Desoxycorticosterone Acetate | C,H,O | 154-159 | — | — | Insoluble | Soluble |
| 157 | Dextrose | C,H,O | — | — | — | Less than 1 | 50 |
| 158 | Diamorphine | C,H,O,N | 169 | — | — | 800 | 44 |
| 159 | Diamorphine Hydrochloride | C,H,O,N, Cl | 233 | — | — | 2.5 | 13 |
| 160 | Dichloramine | C,H,O,N, Cl,S | 78 | — | — | Almost insoluble | Soluble with decomp. |
| 161 | <i>o</i> -Dichlorobenzene | C,H,Cl | — | 179 | 1.327 | Almost insoluble | Soluble |
| 162 | <i>p</i> -Dichlorobenzene | C,H,Cl | 53-54 | 172- 174 | — | Insoluble | Soluble |
| 163 | Digitalin | C,H,O | — | — | — | Readily | Readily |
| 164 | Digitoxin | C,H,O | Not below 240 | — | — | Insoluble | 140 |
| 165 | Digoxin | C,H,O | 265 | — | — | Insoluble | Soluble |
| 166 | Diodone (Solution) | C,H,O,N, I | — | — | 1.19 | — | — |

| No. | SPECIAL TESTS |
|-----|---|
| 155 | Heavy, inflammable gas; mixtures with oxygen or air are explosive. |
| 156 | Specific rotation in 1% <i>w/v</i> sol. in dehydrated alcohol, +175° to 185°. |
| 157 | Specific rotation of well-boiled 10% aqueous solution about +52°. Reduces silver nitrate solution on warming. In addition to Fehling's, Barfoed's reagent (warm) is also reduced (distinction from dextrin and maltose). Sodium, calcium and barium oxides form saccharates soluble in water. Ferments with yeast. |
| 158 | Gives yellowish-red with sulphuric acid and a little nitric acid darkening on warming. From acid solutions is pptd. by caustic alkalis, ammonia and ammonium carbonate, re-dissolved by the first in excess. Does not reduce iodates (distinction from morphine). <i>See also</i> the hydrochloride. |
| 159 | Dissolve 0.05 g. in 5 ml. water; add 3 drops 5% ferric chloride solution; no blue colour (distinction from morphine). Nitric acid dissolves with yellow colour changing to blue on warming and becoming yellow again on cooling. Solution in conc. sulphuric acid, warmed, cooled, diluted with water and treated with potassium ferricyanide solution containing a trace of ferric chloride gives deep blue colour. Morphine, ethyl-morphine and codeine do not yield this reaction. |
| 160 | Ppt. with proteins. Soluble in alcohol, ether and cottonseed oil, but decomposes them. Readily soluble in chloroform and eucalyptus oil without evident change. Dissolves in solutions of fixed alkalis forming the soluble monochloramine. Strong oxidising agent liberating iodine from potassium iodide. Gives no ppt. with mercuric chloride (<i>cf.</i> chloramine). |
| 161 | Colourless liquid with characteristic odour. |
| 162 | Mono-nitro derivative has m.p. 55°. |
| 163 | 1 mg. in 1 ml. glacial acetic acid containing trace of ferric chloride layered on to sulphuric acid gives reddish band. Usually adjusted to standard strength by admixture with lactose. |
| 164 | Dissolves in cold hydrochloric acid giving colourless solution which becomes brown on warming. To 0.1 mg. in Petit's liquor add 2 ml. of acetic acid and 1 drop of ferric chloride solution, and layer on to sulphuric acid; a brown ring, blue-green above, is produced. |
| 165 | Specific rotation in 2% <i>v/v</i> sol. in pyridine (Hg light) +13.5 to 13.7. 1 ml. of 0.1% sol. in glacial acetic acid containing 0.01% FeCl ₃ with layer of conc. H ₂ SO ₄ gives brown ring and the acetic acid layer acquires an indigo colour. |
| 166 | Usually in aqueous solution, containing about 35% of hygroscopic diethanolamine salt of diiodine and about 17% <i>v/v</i> of iodine. |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|-------------------------------------|------------------|--|-------------|------------|---------------------------------|---------------------------------------|
| 167 | Diphenan | C,H,O,N | 146-150 | — | — | Insoluble | Soluble |
| 168 | Disodium Methyl- arsonate | C,H,O,As, Na | — | — | — | 1 | 350 |
| 169 | Dithranol | C,H,O | 174-178 | — | — | Insoluble | Slight |
| 170 | Dulcin | C,H,O,N | 172-174 | — | — | Slight | Soluble |
| 171 | Elaterin | C,H,O | 209 | — | — | Insoluble | 160 |
| 172 | Emetine | C,H,O,N | About 74 | — | — | Slight | 3 |
| 173 | Emetine Hydro- chloride | C,H,O,N, Cl | — | — | — | 9 | Soluble |
| 174 | Emetine and Bismuth Iodide | C,H,O,N, I,Bi | — | — | — | Insoluble | Insoluble |
| 175 | Ephedrine | C,H,O,N | About 40 | — | — | Readily | Readily |
| 176 | Ephedrine Hydro- chloride | C,H,O,N, Cl | 217-220 | — | — | | About 5 |
| 177 | Ergometrine | C,H,O,N | 162-164 crystall- ised f'm acetone 212 | — | — | Slight | Soluble |
| 178 | Ergometrine Tartrate | C,H,O,N | — | — | — | Soluble | Soluble |
| 179 | Ergotoxine | C,H,O,N | Chars at 150-160 | — | — | Very slight | Soluble |
| 180 | Ergotoxine Ethane- sulphonate | C,H,O,N, S | Chars at 170-180 | — | — | Soluble | Soluble |
| 181 | Erythrityl Tetra- nitrate | C,H,O,N | 61 | — | — | Insoluble | About 90 |

| No. | SPECIAL TESTS. |
|-----|---|
| 167 | Heated with H_2SO_4 until brown and then with excess of NaOH , ammonia is evolved. |
| 168 | 0.0005 g. in 5 ml. water gives white ppt. with silver nitrate, a violet ppt. with cobalt nitrate and a white ppt. on warming with calcium chloride (distinction from sodium cacodylate). |
| 169 | Gives yellow or red solution in dilute NaOH with intense green fluorescence. |
| 170 | Sulphur is absent (distinction from saccharin). |
| 171 | With Froehde's reagent, first green then brown colour. Mandelin's reagent gives blue. A solution of about 0.01 g. in 5 ml. of melted phenol becomes crimson, changing to scarlet on adding a few drops of sulphuric acid. |
| 172 | Sulphomolybdic acid gives green colour with the hydrochloride or in presence of trace of chloride. 0.0002 g., e.g. in Tinct. Ipecac., gives yellow with hydrochloric acid and hydrogen peroxide. |
| 173 | Gives reactions of emetine and chlorides. Sulphomolybdic acid gives green colour. |
| 174 | Gives reactions for bismuth and iodine. Quickly loses its red colour on shaking with sodium bicarbonate and (more slowly) with 0.2% hydrochloric acid. |
| 175 | 0.01 g. in 1 ml. water with 0.1 ml. aqueous solution of copper sulphate and 1 ml. sodium hydroxide gives violet colour; on shaking mixture with ether, latter becomes purple and aqueous solution blue. Gives the hydrochloride on standing in contact with chloroform and evaporating. 0.01 g. in 1 ml. water with 1 ml. potassium ferricyanide solution and 1 ml. potassium hydroxide gives benzaldehyde odour. |
| 176 | Gives reaction for hydrochloride, and with copper sulphate reacts as ephedrine. |
| 177 | The dilute aqueous solution gives a blue colour with solution of dimethylaminobenzaldehyde. A purple colour is produced on adding 2 drops of sulphuric acid to 1 ml. of a 0.1% solution in glacial acetic acid containing a trace of ferric chloride. Crystals may contain solvent of crystallisation. |
| 178 | Gives reactions of ergometrine and tartaric acid. |
| 179 | Gives the same reactions as ergometrine. |
| 180 | Gives the same reactions as ergometrine. The acid radicle can be determined by dissolving in methyl alcohol, diluting and titrating with N/10 potassium hydroxide using phenolphthalein. |
| 181 | Usually occurs mixed with lactose from which it can be separated by extraction with alcohol. The residue obtained on evaporating the alcohol explodes on percussion. |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|---------------------------------------|----------------|------------------------------|----------------------|--|---------------------------------|---------------------------------------|
| 182 | Ethyl Acetate | C,H,O | — | 77 (when pure) | 0.900— 0.907 (0.924 when pure) | 15 | Miscible |
| 183 | Ethyl Hydroxy- benzoate | C,H,O | 113 | — | — | Slight | Soluble |
| 184 | Ethyl Phthalate | C,H,O | — | 280— 300 | About 1.12 | Insoluble | Miscible |
| 185 | Ethylmor- phine Hydro- chloride | C,H,O,N, Cl | About 123 | — | — | 10 | 25 |
| 186 | Eucalyptol | C,H,O | — | 176— 177 | 0.928— 0.930 | Insoluble | 2 |
| 187 | Euflavine | C,H,N,Cl | — | — | — | Slight | Slight |
| 188 | Fluorescein (Soluble) | C,H,O,Na | — | — | — | Less than 1 | 5 |
| 189 | Formalde- hyde Solution | C,H,O | — | — | 1.080— 1.095 | Miscible | Miscible |
| 190 | Fuchsine | C,H,O,N, Cl | — | — | — | Slight | Soluble |
| 191 | Gelsemine | C,H,O,N | About 178 | — | — | Slight | Soluble |
| 192 | Guaiacol | C,H,O | 28(crys- talline form) | 200— 210 | 1.116— 1.125 (liquid form) | 80 | Miscible |
| 193 | Guaiacol Benzoate | C,H,O | 50-52 | — | — | Almost insoluble | 50 |
| 194 | Guaiacol Carbonate | C,H,O | 85-88 | — | — | Insoluble | About 200 |

| No. | SPECIAL TESTS |
|-----|---|
| 182 | Characteristic fragrant odour. Yields ethyl alcohol and acetic acid when hydrolysed. |
| 183 | A trace boiled with H_2O gives reddish-violet solution with 1 drop of ferric chloride changing to brown on adding further 2 drops. Mercuric nitrate to boiling solution gives ppt. and red supernatant liquid. |
| 184 | Gives phthalic acid and ethyl alcohol on hydrolysis. |
| 185 | Does not give blue colour with ferric chloride or reduce iodates directly. 0.01 g. dissolved in 10 ml. sulphuric acid after liberation of the hydrochloric acid, gives a clear solution, which on adding a drop of ferric chloride solution and warming turns violet or blue, changing to red on adding 2 to 3 drops of nitric acid. The free base is less soluble in ammonia than codeine. Such solution reprecipitates the base in crystals melting at 93° . |
| 186 | 1 ml. in a freezing mixture with equal volume of phosphoric acid added gradually gives a white crystalline mass of eucalyptol phosphate. If warm water is then added eucalyptol will separate. Agitated with strong solution of iodine in potassium iodide a pasty mass is produced in which green lustrous crystals are formed. |
| 187 | Bulky yellow ppt. on adding sodium salicylate to aqueous solution. No effervescence with sodium bicarbonate (<i>cf.</i> acriflavine). No ppt. with solution of formaldehyde (<i>cf.</i> proflavine). |
| 188 | Unmistakeable fluorescence in solution. Colour discharged by acid. Heated with zinc dust and sodium hydroxide reduced to colourless fluorescin. |
| 189 | Combines with ammonia. Reduces ammoniacal silver nitrate solution (mirror). Responds to Schiff's reagent. <i>See also</i> Milk Tests, Urine Tests, and Paraformaldehyde. |
| 190 | Is decolorised by zinc and hydrochloric acid also by sulphurous acid. For detection of minute quantities, as in urine, extract with acetic ether or amyl alcohol. The colour in these disappears on adding ammonia or hydrochloric acid if fuchsine present. |
| 191 | 0.001 g. gives green colour with conc. nitric acid. 0.001 g. with sulphuric acid and potassium dichromate gives reddish-violet turning green. |
| 192 | Occurs in crystalline form, or as a liquid. Characteristic odour and taste. An alcoholic solution with ferric chloride gives emerald-green changing to blue and then brown. Gives yellow and not red-brown on shaking with 10 vols. of sulphuric acid (distinction from creosote). |
| 193 | When hydrolysed gives reactions for guaiacol and benzoic acid. |
| 194 | 0.01 g. gives guaiacol and potassium carbonate on hydrolysis with alcoholic potash. |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|------------------------------------|----------------|--|-------------|------------|---------------------------------|---------------------------------------|
| 195 | Guaiacol Cinnamate | C,H,O | 130 | — | — | Insoluble | Soluble |
| 196 | Hexamine | C,H,N | Sub- limes with dec'mp. at 263 | — | — | 1·5 | 8 |
| 197 | Hexobar- bitone | C,H,O,N | 145-147 | — | — | Slight | Soluble |
| 198 | Hexobar- bitone Soluble | C,H,O,N, Na | — | — | — | Very soluble | Soluble |
| 199 | Hexylresor- cinol | C,H,O | About 66 | — | — | 2000 | Readily |
| 200 | Histamine | C,H,N | — | — | — | Soluble | Soluble |
| 201 | Histamine Acid Phosphate | C,H,O,N, P | 130-133 after sinter- ing at 127 | — | — | 4·5 | Slight |
| 202 | Homatropine Hydro- bromide | C,H,O,N, Br | 214 | — | — | 6 | 18 |
| 203 | Hydrastine Hydro- chloride | C,H,O,N, Cl | 168 when dried | — | — | Soluble | 120 |
| 204 | Hydrastinine Hydro- chloride | C,H,O,N, Cl | About 210 | — | — | Less than 1 | 3 |
| 205 | Hyoscine Hydro- bromide | C,H,O,N, Br | 194-196 when dried | — | — | 2 | 13 |
| 206 | Hyoscyamine Sulphate | C,H,O,N, S | 203-206 | — | — | 0·5 | 4·5 |

| No. | SPECIAL TESTS |
|-----|--|
| 195 | When hydrolysed gives reactions for guaiacol and cinnamic acid. |
| 196 | Boiled with dilute acids gives formaldehyde and ammonium salt. Gives ppt. with mercuric chloride solution becoming crystalline on standing. |
| 197 | Dissolves in aqueous alkali hydroxides. Heat with aq. Na_2CO_3 on water-bath with alcoholic sol. of nitrobenzyl chloride for 30 minutes and let stand 1 hour; wash ppt. with dilute NaOH and recryst. from light petroleum; the m.p. of product is 116° – 117° . |
| 198 | Sol. in H_2O gives ppt. of hexobarbitone on adding acid. |
| 199 | Paraffin odour on heating. Sulphuric acid cold dissolves, hot chars and sulphur dioxide is evolved. Nitric acid gives violent reaction. Trace of ferric chloride added to alcoholic solution gives green colour. |
| 200 | Gives Pauly's reaction—a rose-pink coloration is obtained on adding sodium <i>p</i> -diazobenzenesulphonate to 1 in 10,000 aqueous solution. |
| 201 | An alkaline solution gives a deep red colour when mixed with an aqueous solution of sulphanilic acid containing a trace of hydrochloric acid and of sodium nitrite. The picrolonate obtained by mixing a hot solution of histamine acid phosphate with a hot alcoholic solution of picrolonic acid, after washing and drying has m.p. 266° to 267° . |
| 202 | Alkaloidal base, obtained by adding ammonia to aqueous solution, shaking out with chloroform and evaporating solvent, melts at about 98° and when warmed with 2% solution of mercuric chloride in alcohol (60%) gives yellow ppt. becoming brick-red. Does not react to Vitali's test—0.0001 g. gives yellow instead of violet given by atropine (<i>q.v.</i>). Does not ppt. with tannic acid or platinic chloride after adding hydrochloric acid. |
| 203 | Froehde's reagent gives green to brown colour. Sulphovanadic acid gives orange-red. Sulphuric acid with trace of molybdic acid gives green colour changing to brown. Sulphuric acid with trace of potassium dichromate gives light green colour changing to brown. |
| 204 | Aqueous solution acidified with sulphuric acid has blue fluorescence. With Nessler's reagent gives black ppt. of mercury. |
| 205 | Response to Vitali's reaction very similar to that of atropine (<i>q.v.</i>). Gold chloride compound obtained by dissolving separated base in hydrochloric acid, adding solution of auric chloride and recrystallising from water, has m.p. of 198° to 200° . |
| 206 | Response to Vitali's reaction very similar to that of atropine (<i>q.v.</i>). Gold salt (recrystallised from hot water) is in golden shining leaflets, m.p. 165° ; solutions reduce in the light. A 1 in 20 solution does not ppt. with platinic chloride solution (distinction from most alkaloids). |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|------------------------------------|----------------------|---------------------------------------|-------------|------------|---------------------------------|--|
| 207 | Indigo | C, H, O, N | — | — | — | Very slight | Insoluble |
| 208 | Indigo Carmine | C, H, O, N, S, Na | — | — | — | 100 | Insoluble |
| 209 | Iodo-caffeine | C, H, O, N, I Na | — | — | — | Soluble | Soluble |
| 210 | Iodoform | C, H, I | 120-122 | — | — | Very slight | 100 |
| 211 | Iodo-phthalein | C, H, O, I, Na | — | — | — | 7 | Slight |
| 212 | Iodopyrrole | C, H, N, I | De- comp. above 140 | — | — | Almost insoluble | 21 |
| 213 | Iodoxyl | C, H, O, N, I, Na | — | — | — | 1.2 | 100 |
| 214 | Lactose | C, H, O | 200 (de- comp.) | — | — | 7 | Almost insoluble |
| 215 | Lævulose | C, H, O | About 95 when anhy- drous | — | — | Less than 0.5 | About 16 |
| 216 | Lecithin (Ovo-) | C, H, O, N, P | — | — | — | Slight | 30 (Mis- cible with 1 but pptd. by more) |
| 217 | Leptazol | C, H, N | 57-60 | — | — | Soluble | Soluble |
| 218 | Lithium Acetyl-salicylate | C, H, O, Li | — | — | — | 1 | 4 |
| 219 | Magnesium Acetyl-salicylate | C, H, O, Mg | — | — | — | 12 | Insoluble |
| 220 | Malachite Green | C, H, O, N | — | — | — | 2 | About 30 |

| No. | SPECIAL TESTS |
|-----|--|
| 207 | Purple vapours on heating in test-tube. Colour disappears on treatment with alkaline reducing agents, <i>e.g.</i> , zinc and sodium hydroxide. |
| 208 | Ash contains sodium sulphate. Diazo test gives green colour. Colour of aqueous solution destroyed by nitric acid or bromine water, or by zinc and sodium hydroxide. |
| 209 | Readily soluble in sodium iodide solution. |
| 210 | Iodine is pptd. on adding nitric acid to a solution in warm alcoholic potassium hydroxide. |
| 211 | Cream ppt. with hydrochloric acid which re-dissolves in excess of sodium hydroxide solution with return of blue colour which subsequently disappears. Fused with sodium hydroxide, dissolved in nitric acid and treated with silver nitrate a yellow ppt. is obtained. |
| 212 | Warmed with zinc and sodium hydroxide, fumes of pyrrole are given off and impart a red colour to pine wood (<i>e.g.</i> a match) soaked in hydrochloric acid. Alcoholic solution gives a red colour with nitric acid. |
| 213 | Contains 50.5 to 52.5% of iodine and 9.2 to 9.4% of sodium. Addition of acid gives ppt. of the acid which melts with decomposition at about 174°. |
| 214 | Loses water at 130° and becomes yellow at 160°. Reduces Fehling's solution on heating. Becomes brown on heating with alkalis. Osazone separates only on cooling after prolonged heating and has m.p. 200° (decomp.). |
| 215 | Reduces bismuth salts in alkaline solution. On warming with alkali hydroxide the solution turns brown (as also dextrose). Fermentable directly but more slowly than dextrose. Combines with calcium hydroxide, forming insoluble gelatinous salt. Specific rotation of well-boiled 10% aqueous solution about -93° if pure. |
| 216 | Characteristic waxy appearance. Boiled with baryta gives glycerophosphoric acid, neurine, and a fatty acid (stearic, oleic, or palmitic). |
| 217 | Mercuric chloride to 10% sol. gives ppt., which, after recrystallising, melts at 177°-178°. |
| 218 | Hydrochloric acid ppts. aspirin; filtrate gives reactions for lithium. After hydrolysis gives reactions of salicylic acid. |
| 219 | Hydrochloric acid precipitates aspirin; filtrate gives reactions for magnesium. After hydrolysis gives reactions for salicylic acid. |
| 220 | Its solubility in amyl alcohol distinguishes it from methyl green and its allies. Aqueous solution becomes red on acidifying with hydrochloric acid. Colour base is pptd. on addition of sodium hydroxide and when recrystallised from benzene has m.p. of about 132°. |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|-------------------------------------|--------------------|--|-------------|---------------|---------------------------------|---------------------------------------|
| 221 | Maltose | C,H,O | De- comp. on heating 113 | — | — | Very soluble | Slightly soluble |
| 222 | Mannityl Hexanitrate | C,H,O,N | — | — | — | Almost insoluble | Slightly soluble |
| 223 | Menaphthone | C,H,O | 104-107 | — | — | Insoluble | Slightly |
| 224 | Menthol | C,H,O | 42-43 | — | — | Almost insoluble | 0.2 |
| 225 | Mepacrine Hydro- chloride | C,H,O,N, Cl | — | — | — | 30 | Soluble |
| 226 | Mepacrine Methane- sulphonate | C,H,O,N, S,Cl | — | — | — | 3 | 36 |
| 227 | Mercurio- chrome | C,H,O,Br, Na,Hg | — | — | — | Readily | Insoluble |
| 228 | Mercury Cyanide | C,N,Hg | — | — | — | 13 | 20 |
| 229 | Mercury Oxycyanide | C,O,N,Hg | — | — | — | 18 | Slight |
| 230 | Mercury Salicylate | C,H,O,N Hg | — | — | — | Almost insoluble | Almost insoluble |
| 231 | Mercury Succini- mide | C,H,O,N, Hg | — | — | — | 20 at 25° | Slight |
| 232 | Mersalyl | C,H,O,N, Na,Hg | — | — | — | 1 | 3 |
| 233 | Mesulphen | C,H,S | De- posits crystals m.p. 123 | — | 1.20- 1.22 | Insoluble | Soluble |
| 234 | Metaldehyde | C,H,O | Sub- limes at 100 | — | — | Insoluble | Almost insoluble |

| No. | SPECIAL TESTS |
|-----|---|
| 221 | Reduces Fehling's solution. Osazone separates on cooling after prolonged heating and has m.p. of 206°. |
| 222 | Explodes at 120°. |
| 223 | Turns light brown and decomposes in sunlight. Treat 0.0005 g. in 5 ml. of alc. with 2 ml. of strong ammonia and a few drops of ethyl cyanoacetate; violet colour is produced changing to yellow on adding 5 ml. of 30% sodium hydroxide. |
| 224 | Heated with benzoic anhydride the benzoic ester is obtained, m.p. 54.5°. Synthetic menthol usually has a lower m.p. |
| 225 | Yellow, bitter, crystalline powder. Ammonia to aqueous solution gives yellow or orange ppt. which is soluble in ether. Dilute nitric acid and mercuric chloride solution give yellow-green fluorescence and yellow precipitate. |
| 226 | Yellow, bitter, crystalline powder. Gives reactions as for Mepacrine Hydrochloride. After fusion with Na_2CO_3 , residue gives reactions for sulphate. |
| 227 | Hydrochloric acid precipitates dibromohydroxymercurifluorescein. Heated with sulphuric acid and nitric acid, cooled and diluted with water gives a ppt. of mercuric sulphide with hydrogen sulphide. Fused with sodium hydroxide and dissolved in dilute nitric acid, gives a ppt. of silver bromide with silver nitrate. |
| 228 | Decomposed on heating into mercury and cyanogen. Hydrogen cyanide evolved when heated with dilute hydrochloric acid. |
| 229 | Not ionised in solution and does not give the usual reactions for mercury and cyanide. |
| 230 | Decomposed by strong mineral acids with precipitation of salicylic acid. |
| 231 | Aqueous solution gives with sodium hydroxide a yellowish-white ppt. reduced to metallic mercury on heating. Heated with zinc dust in excess, pyrrole is evolved and imparts a red colour to pinewood (e.g. a match) moistened with hydrochloric acid and held in the vapour. |
| 232 | When heated for 15 minutes under a reflux condenser with formic acid and water (1 : 1) and the liquid decanted and cooled, salicylaldehyde-O-acetic acid separates; after washing and drying <i>in vacuo</i> crystals have m.p. 119° to 121°. |
| 233 | Boiling range 130° to 200° at 3 mm. pressure; R.I. at 55° about 1.66. Dissolved in H_2SO_4 gives intense violet-blue colour which disappears on dilution. Contains about 23 to 26% of sulphur. |
| 234 | Heated with dilute sulphuric acid, acetaldehyde is obtained. |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|---|---------------------|--------------------------------|--------------|-----------------|---------------------------------|---------------------------------------|
| 235 | Methyl Hydroxy- benzoate | C,H,O | 120-130 | — | — | 400 | 3 |
| 236 | Methyl Salicylate | C,H,O | — | 224 | 1.186- 1.191 | Slight | Miscible |
| 237 | Methylacet- anilide | C,H,O,N | 100-101 | — | — | 60 | Readily |
| 238 | Methylene Blue | C,H,N,Cl S | — | — | — | About 6 | Slight |
| 239 | Methylene- ditannin | C,H,O | 220-240 (de- comp.) | — | — | Insoluble | About 3 |
| 240 | Methylsul- phonol | C,H,O,S | 76-78 | — | — | 320 | 12 |
| 241 | Morphine | C,H,O,N | About 230 (de- comp.) | — | — | 5000 | 100 |
| 242 | Morphine Hydrochloride | C,H,O,N, Cl | — | — | — | 25 | About 50 |
| 243 | Naphthalene | C,H | 79-80 | About 218 | — | Insoluble | 23 |
| 244 | Narcotine | C,H,O,N | 175-176 | — | — | Insoluble | Soluble |
| 245 | Neoarsphen- amine | C,H,O,N, S,Na,As | — | — | — | Soluble | Insoluble |
| 246 | Neocincho- phen | C,H,O,N | 76 | — | — | Insoluble | Soluble |
| 247 | Nicotine | C,H,N | — | 240- 246 | About 1.01 | Miscible | Miscible |

| No. | SPECIAL TESTS |
|-----|---|
| 235 | A trace boiled with H_2O gives reddish-violet solution with 1 drop of ferric chloride changing to brown on adding further 2 drops. Mercuric nitrate to boiling solution gives ppt. and red supernatant liquid. |
| 236 | Characteristic odour. Gives violet colour with ferric chloride. When hydrolysed with alkali and the acid precipitated by adding hydrochloric acid, salicylic acid, m.p. 158° , is obtained. |
| 237 | Is decomposed on heating with sodium hydroxide or sulphuric acid into acetic acid and monomethylaniline. The latter gives with nitrous acid a nitroso compound insoluble in sodium hydroxide. Soluble in chloroform (1 in 2)—distinction from acetanilide and phenacetin. |
| 238 | Aqueous solution decolorised on warming with zinc dust and acetic acid, colour reappearing if solution is filtered and exposed to air. Aqueous solution with potassium iodide solution gives deep blue flocculent ppt. Aqueous solution with slightly acid solution of potassium dichromate gives bluish-violet ppt., the liquid becoming red-violet but the blue is restored with sulphurous acid. Nitrous acid converts to methylene green. |
| 239 | 0.1 g., heated with 2 ml. conc. sulphuric acid, gives a brown colour changing to green and then blue, and on adding alcohol a blue turning to wine-red. |
| 240 | Heated with anhydrous sodium acetate, hydrogen sulphide is evolved. Heated with charcoal gives odour of mercaptan. |
| 241 | Sprinkled on nitric acid gives orange-red colour. Heated with sulphuric acid on a water-bath for 15 minutes, cooled and treated with nitric acid gives blood-red colour. Froehde's reagent gives reddish-violet, changing to slate blue. Liberates iodine from iodic acid. Gives blue ppt. with ferric chloride and potassium ferricyanide solution (freshly prepared). |
| 242 | Gives reactions of morphine and hydrochloride. |
| 243 | Characteristic odour. The picrate, precipitated from alcoholic solution, has m.p. 150° . |
| 244 | Dissolves in conc. sulphuric acid giving greenish-yellow solution becoming red on warming and violet on boiling. Stirred with conc. sulphuric acid and a crystal of potassium dichromate an intense red coloration is produced. |
| 245 | On adding hydrochloric acid to aqueous solution, a yellow ppt. of free acid is produced; if mixture is heated, sulphur dioxide is evolved. On distilling the aqueous solution acidified with phosphoric acid, the distillate contains formaldehyde. |
| 246 | Excess of bromine water added to the solution in sulphuric acid gives a yellow ppt. Ferric chloride solution added to the 1% solution in alcohol gives a yellow coloration (cinchophen gives a reddish-brown). |
| 247 | Characteristic odour. Has properties of a tertiary amine. The picrate has m.p. 219° . A rose-red coloration is produced on adding 1 drop of formaldehyde solution followed by 1 drop of nitric acid. |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|-----------------------|----------------|-----------------------------|-------------|-----------------|---------------------------------|---------------------------------------|
| 248 | Nikethamide | C,H,O,N | 22-24 | — | 1.064- 1.067 | Soluble | Soluble |
| 249 | Nitro- benzene | C,H,O,N | About 5 | 209- 211 | 1.21 | Almost insoluble | Miscible |
| 250 | Nupercaine | C,H,O,N, Cl | 97 | — | — | Soluble | Soluble |
| 251 | Œstradiol | C,H,O | 175-178 | — | — | Insoluble | Soluble |
| 252 | Œstradiol Benzoate | C,H,O | 189-195 | — | — | Insoluble | Soluble |
| 253 | Œstriol | C,H,O | About 282 | — | — | Insoluble | Soluble |
| 254 | Œstrone | C,H,O | 255-260 | — | — | Insoluble | Soluble |
| 255 | Orthocaine | C,H,O,N | 141-143 | — | — | Slight | 7 |
| 256 | Ouabain | C,H,O | 187-188 (when anhyd.) | — | — | 100 | 30 (dehy- drated alcohol) |
| 257 | Pamaquin | C,H,O,N | — | — | — | Insoluble | Soluble |
| 258 | Papaverine | C,H,O,N | 146-147 | — | — | Insoluble | Slight |
| 259 | Paraform- aldehyde | C,H,O | Volatil- ises at 100 | — | — | Insoluble | Slight |

| No. | SPECIAL TESTS |
|-----|---|
| 248 | Oily liquid or crystalline solid. R.I. at 20°, about 1.525. The 25% solution gives ppt. with alkaline potassio-mercuric iodide or mercuric chloride, and brownish flocculent ppt. with tannic acid, but no ppt. with potassio-mercuric iodide or picric acid. |
| 249 | Detection of traces: distil with a little sulphuric acid in steam, shake distillate with chloroform, convert oily drops into aniline by reduction with zinc and sulphuric acid. |
| 250 | Anæsthetic (surface, spinal, etc.), formerly known as Percaine. Gives reactions for chlorides unless in form of free base. |
| 251 | Specific rotation of 1% solution in dioxan +75° to 82°. Monobenzoate melts at about 190° and dipropionate at about 103°. |
| 252 | Specific rotation of 1% solution in dioxan +58° to 61°. Alkalis liberate oestradiol, which melts at 175°-178°. |
| 253 | Specific rotation of 1% solution in dioxan +53° to 63°. |
| 254 | Specific rotation of 1% solution in dioxan +157° to 167°. Monomethyl derivative melts at about 165°; monoacetate, about 125°; semicarbazone, about 258°; benzoate, 218° to 222°. |
| 255 | 5% aqueous solution with 10% sodium nitrite solution acidified with hydrochloric acid gives yellowish-white and on exposure to air an orange ppt. darkening to red. Distinguished from amydracaine, amylocaine, benzamine, cocaine, phenacaine, procaine and tropacocaine by Froehde's reagent which gives a faint violet tinge but nil with others except tropacocaine which gives slight green. Gives no ppt. with solution of iodine (distinction from benzocaine). |
| 256 | 1 ml. of 1% aqueous solution layered on to conc. sulphuric acid acquires a dirty green coloration and the acid is coloured pink to red. |
| 257 | Yellow, or orange-yellow, bitter powder. Formaldehyde added to 1% mixture with sulphuric acid gives green colour slowly. Add HCl to 4% solution in acetone, filter off white ppt., after adding water and then add powdered sodium iodate; violet colour appears suddenly after about 2 minutes. |
| 258 | 0.01 g. with 5 ml. of sulphuric acid gives colourless solution becoming rose-red at 110° and darkening to violet at 200°, the colour being discharged on adding water. Gives rose-red colour with sulphuric acid containing solution of formaldehyde. Aqueous solution of potassium ferricyanide gives with a faintly acid solution a yellow ppt. which when washed and dried dissolves in sulphuric acid containing solution of formaldehyde, giving a pale blue colour darkening to bluish-violet. |
| 259 | Formaldehyde odour on heating. Distillate in water reduces silver nitrate (forming mirror). Responds to Schiff's test (sulphurous fuchsin solution). 0.5% sodium nitroprusside solution gives red coloration changing to purple on acidifying with acetic acid. Nessler's reagent gives a reddish ppt. which gradually changes to grey. If to 5 ml. sulphuric acid in which 0.02 g. salicylic acid is dissolved, 2 drops of formaldehyde (37%) be added and the liquid gently warmed, a permanent red colour forms. In general, gives the reactions of aldehydes. |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|--|----------------|----------------------------|-------------|-----------------|---------------------------------|---------------------------------------|
| 260 | Paraldehyde | C,H,O | 11-12 | 123- 126 | 0.998- 1.000 | 9 | Miscible |
| 261 | Pectin | C,H,O | — | — | — | Soluble | Insoluble |
| 262 | Pelletierine | C,H,O,N | — | 195 | 0.988 | 23 | Miscible |
| 263 | Pelletierine Tannate | C,H,O,N | Softens at about 165 | — | — | 700 | 80 |
| 264 | Pentobarbi- tone Soluble (Nembutal) | C,H,O,N, Na | — | — | — | Soluble | Freely |
| 265 | Phemitone | C,H,O,N | 178-181 | — | — | Insoluble | Soluble |
| 266 | Phenacaine Hydro- chloride | C,H,O,N, Cl | 186-189 | — | — | 55 | 8 |
| 267 | Phenacetin | C,H,O,N | 134-136 | — | — | 1700 | 21 |
| 268 | Phenazone | C,H,O,N | 111-113 | — | — | 1.25 | 1.3 |
| 269 | Phenobar- bitone | C,H,O,N | 173-177 | — | — | 1000 | 15 |

| No. | SPECIAL TESTS |
|-----|---|
| 260 | More soluble in cold water than hot—saturated aqueous solution becomes turbid on warming. Gives mirror with ammoniacal silver nitrate on warming. Gives reactions of aldehydes, but does not add ammonia or sodium bisulphite. Warmed with sulphuric acid it is converted into acetaldehyde. |
| 261 | Neutral complex carbohydrate reducing Fehling's solution. Contains about 11.8% of methyl alcohol as methoxyester. Yields calcium pectate after hydrolysis containing 7.62% of calcium. |
| 262 | A liquid alkaloid giving a strongly alkaline solution. On reduction it yields coniine. Hydrochloride has m.p. 143° to 144°; hydrobromide has m.p. 140°. |
| 263 | Gives deep blue ppt. with cobalt or copper sulphate. The base absorbs oxygen from the air, resinifying. Pelletierine with sulphuric or selenious acid gives a red colour, deepening on heating and changing to green (distinction from arecoline). |
| 264 | Isomeric with amylethylbarbitone soluble; contains methylbutyl group instead of iso-amyl. Gives general reactions of malonylureas. |
| 265 | Soluble in solutions of alkali hydroxides. Ammonia and methylamine evolved when heated with alkali hydroxide. |
| 266 | Yellow waxy ppt. with sodium nitrite and hydrochloric acid. |
| 267 | Potassium dichromate in hydrochloric acid solution gives red colour. 1 ml. of a solution of 0.2 g. in 2 ml. hydrochloric acid (25%) boiled cooled and filtered gives reddish violet on adding 5 drops chlorine water and violet changing to red on adding 1 drop potassium dichromate solution. Colour reaction (Carletti): moisten a small quantity of phenacetin in a dish with acetaldehyde and 2 to 3 ml. sulphuric acid; on stirring with a rod the acid turns red (increased by warming). Sensitive to 0.001 g. |
| 268 | Aqueous solution with trace of sodium nitrite and dilute sulphuric acid gives green colour. 1 to 2 ml. of 0.1% aqueous solution with 1 drop of ferric chloride solution gives deep red colour changed to light yellow by dilute sulphuric acid. Aqueous solution gives with an equal volume of nitric acid a yellow solution changing to crimson on warming. Tannin gives a white ppt. with 1% solution. |
| 269 | TO DISTINGUISH FROM BARBITONE. Dissolve 0.1 g. in 1 ml. sulphuric acid and add trace of sodium nitrite—orange colour with phenobarbitone, none with barbitone. Heat with sodium hydroxide, acidify with sulphuric acid—carbon dioxide evolved with sweet acetic odour. Gives ppt. with Millon's reagent, with or without nitric acid. Mix 0.1 g. with 0.5 g. potassium nitrate and 2 ml. sulphuric acid. Heat on boiling water-bath 10 minutes and pour into 10 ml. cold water. Reduce pptd. nitro-compound with Zn and decant solution. Cool, acidify, add aqueous solution of 0.1 g. potassium nitrite and add to a solution of β -naphthol in sodium hydroxide—blood-red colour distinguishes barbituric acid with phenyl group. |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|--------------------------------------|----------------------|---|-------------|------------|-----------------------------------|---------------------------------------|
| 270 | Phenobarbitone Soluble | $C_8H_8O_3N, Na$ | — | — | — | Readily | Readily |
| 271 | Phenocoll Hydrochloride | $C_8H_8O_3N, Cl$ | — | — | — | 16 | 34 |
| 272 | Phenol | C_6H_6O | 39–40 | — | — | 12 | 0·16 |
| 273 | Phenolphthalein | $C_{20}H_{14}O_4$ | 254–258 | — | — | Insoluble | 10 |
| 274 | m-Phenylenediamine | $C_6H_8N_2$ | 63 | 283 | — | Readily | Readily |
| 275 | p-Phenylenediamine | $C_6H_8N_2$ | 140 | 267 | — | Readily | Readily |
| 276 | Phenylhydrazine Hydrochloride | $C_6H_8N_2, Cl$ | — | — | — | Soluble | Soluble |
| 277 | Phenylmercuric Nitrate | $C_6H_5O_2N, Hg$ | 185–190 (Decomp.) | — | — | Slightly (boiling 1 in 160) | About 1000 |
| 278 | Phenytol Sodium | $C_6H_5O_2N, Na$ | — | — | — | Soluble | Slight |
| 279 | Phloridzin | $C_{25}H_{34}O_{10}$ | 107 solidifies and ag'n melts at 170 | — | — | Almost insoluble | 4·5 |
| 280 | Physostigmine | $C_{11}H_{15}O_3N$ | 105–106 | — | — | Slight | 2 |
| 281 | Physostigmine Salicylate | $C_{11}H_{15}O_3N$ | 140 | — | — | Less than 1 | Less than 1 |
| 282 | Picrotoxin | $C_{21}H_{34}O_8$ | About 200 | — | — | Slight | 13·5 |

| No. | SPECIAL TESTS |
|-----|--|
| 270 | Gives reactions of sodium and yields ppt. of phenobarbitone on acidifying the aqueous solution. |
| 271 | 1 ml. of solution of 0.2 g. in 2 ml. of hydrochloric acid (25%), boiled, cooled and filtered, with 5 drops of fresh chlorine water, gives reddish-violet colour. 0.1 g. boiled with 2 ml. 33% sodium hydroxide and then 2 drops of chloroform added gives phenyl isocyanide odour and black drops on surface. |
| 272 | To 0.001 g. in 10 ml. water add 1 drop of 10% sodium nitrite solution and pour on to sulphuric acid. A coloured zone—red above, green below—appears at the junction. Bleaching powder to an aqueous solution gives violet colour. |
| 273 | Red colour with caustic alkalis disappearing with acids. Silver nitrate gives a violet ppt. |
| 274 | Acidified solution gives coloured ppt. with solution of sodium nitrite. |
| 275 | A trace dissolved in water gives with ferric chlorid a green colour rapidly becoming brown. <i>p</i> -phenylene diamine give ^c with a reagent containing cobalt nitrate 5 g. and ammonium thiocyanate 1 g. in 10 units of water, brown spherical crystals; <i>p</i> -toluylene ^a diamine, 1 : 2 : 5-diaminoanisole and <i>p</i> -amino-diphenylamine do not react. |
| 276 | Reduces Fehling's solution in cold. 1 in 50,000 stated to be detected by following test:—To solution add a few drops of aqueous trimethylamine and several drops of sodium nitroprusside. A blue-green colour is produced becoming more marked on addition of potassium hydroxide and red if heated afterwards. |
| 277 | Sodium sulphide to cold solution gives white ppt., changing to black on boiling. Heated with zinc, reduced iron and NaOH solution, ammonia is evolved. Heated with iodine solution and then decolorised with sodium thiosulphate, gives aromatic odour. |
| 278 | Acidify solution with HCl; the white ppt. of phenytoin after washing and drying melts at 295°. |
| 279 | Solutions have avidity for ammonia. In taking up 10% it turns red and melts to a colourless mass. Mix 0.1 g. with a crystal of vanillin and 1 drop of hydrochloric acid, on warming a red colour is obtained. |
| 280 | See under physostigmine salicylate. |
| 281 | Warmed with a few drops of ammonia a yellowish-red colour is produced, and on evaporation a blue residue giving a blue alcoholic solution. On adding acetic acid the solution is blue by transmitted light with a red fluorescence intensified on dilution with water. |
| 282 | Mixed with thrice its weight of potassium nitrate, moistened with sulphuric acid and then sodium hydroxide in excess added, a red colour is produced (Langley's reaction). On the addition of 1 drop 20% anisaldehyde in dehydrated alcohol to a trace of picrotoxin moistened with sulphuric acid, a violet colour is produced. |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|--|---------------------------|-------------------------------------|---------------------------------|------------|---------------------------------|---------------------------------------|
| 283 | Pilocarpine | C,H,O,N | — | 260 (5 mm. pres- sure) | — | Soluble | Soluble |
| 284 | Pilocarpine Nitrate | C,H,O,N | 174-178 | — | — | 9 | 50 |
| 285 | Piperazine | C,H,N (anhyd- rous) | 43-44; 108-110 when anhyd. | — | — | 2 | 3 |
| 286 | Podophyllo- toxin | C,H,O | 117 | — | — | Almost insoluble | Readily |
| 287 | Potassium Antimonyl- tartrate | C,H,O,K, Sb | — | — | — | 17 | Insoluble |
| 288 | Potassium Guaiacol- sulphonate | C,H,O,S K | — | — | — | Readily | Almost insoluble |
| 289 | Potassium Hydroxy- quinoline Sulphate | C,H,O,N, S,K | Partly lique- fies at 172 | — | — | 1 | Slight |
| 290 | Procaine Hydro- chloride | C,H,O,N, Cl | 154-156 | — | — | 1 | 8 |
| 291 | Proflavine | C,H,O,N, S | — | — | — | 300 | 48 |
| 292 | Progesterone | C,H,O | 128-131 | — | — | Slight | 5 |
| 293 | Propional | C,H,O,N | 145 | — | — | Insoluble | Slight |

| No. | SPECIAL TESTS |
|-----|---|
| 283 | Hydrochloride has m.p. 204° to 205°; aurichloride, 117° to 130° and picrate 147°. |
| 284 | To 2 to 3 ml. of 0.2% aqueous solution acidified with 1 or 2 drops dilute sulphuric acid add equal volume of hydrogen peroxide solution and pour layer of benzene on to the mixture; add 1 or 2 drops of potassium dichromate solution and shake. Benzene layer becomes coloured bluish-violet, the aqueous layer remaining yellow (distinction from other alkaloids). |
| 285 | Dissolves uric acid forming the neutral urate. Gives white ppt. with Nessler's reagent. Piperazine phosphate forms 4-sided tabular crystals. |
| 286 | Alkalis convert into gelatinous acid which loses water, giving a substance melting at 227°. |
| 287 | 0.00005 g. in 5 ml. acidified water gives orange colour with hydrogen sulphide. Larger amount gives ppt. soluble in ammonium sulphide or potassium hydroxide. With Marsh Test—black mirror insoluble in sodium hypochlorite solution. With lime water—white ppt. soluble, when freshly pptd., in acetic acid and ammonium chloride. |
| 288 | Aqueous solution gives intense violet-blue colour with ferric chloride, the colour disappearing on addition of ammonia or strong solutions of alkali sulphates or chlorides. Sulphuric acid and a trace of formaldehyde added to the aqueous solution give a reddish-violet colour. Nitric acid added to the aqueous solution produces a deep red colour on warming; white crystals separate on cooling. |
| 289 | Diazo test gives slight brown-red. Aqueous solution with ferric chloride gives bright green colour. Bromine water gives ppt. of dibromohydroxyquinoline. Gives ppt. with salts of some metals. |
| 290 | Anhydrous base recrystallised from light petroleum has m.p. 58° to 60°. Aqueous solution with trace of sodium nitrite and hydrochloric acid, added to solution of β -naphthol in dilute sodium hydroxide gives scarlet ppt. Slightly acid solution immediately decolorises permanganate solution (distinction from cocaine). Aqueous solution with aqueous solutions of sodium nitrite and sodium carbonate containing 0.5% potassium guaiacolsulphonate, acidified with hydrochloric acid, gives a red to yellow colour, varying with the concentration. |
| 291 | Solutions are similar to those of acriflavine. Gives reactions of sulphates. Aqueous solution reduces permanganate readily and gives ppt. with solution of formaldehyde. |
| 292 | The dioxime has melting-point of 243°. Specific rotation, 1% solution in dehydrated alcohol, +183° to 194° |
| 293 | After heating with sodium hydroxide and acidifying with sulphuric acid, carbon dioxide is evolved and a pungent vapour with odour resembling menthol. Saturated solution gives white ppt. with Millon's. No colour with Nessler's reagent except on fusing with potassium hydroxide. |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|----------------------------------|------------------|----------------------------|-------------|------------|---|---------------------------------------|
| 294 | Propyl Hydroxybenzoate | C,H,O | 96-97 | — | — | 2000 | 2 |
| 295 | Pyridine | C,H,N | — | 115 | 0.98 | Miscible | Miscible |
| 296 | Pyrogallol | C,H,O | 129-135 | — | — | 2 | 1 |
| 297 | Quinidine | C,H,O,N | About 168 | — | — | Almost insoluble | 17 |
| 298 | Quinidine Sulphate | C,H,O,N, S | About 200-202 (decomp.) | — | — | 90 | 10 |
| 299 | Quinine | C,H,O,N | About 174 when anhyd. | — | — | Almost insoluble | 1 |
| 300 | Quinine Acetyl-salicylate | C,H,O,N | 157 | — | — | 330 | 50 |
| 301 | Quinine Bisulphate | C,H,O,N, S | — | — | — | 10 | 23 |
| 302 | Quinine Dihydrochloride | C,H,O,N, Cl | — | — | — | 0.6 | 12 |
| 303 | Quinine Ethyl Carbonate | C,H,O,N | About 90 | — | — | Slight | 2 |
| 304 | Quinine Hydrobromide | C,H,O,N, Br | — | — | — | 55 | .07 |
| 305 | Quinine Iodobismuthate | C,H,I,O, N,Bi | — | — | — | Insoluble, decomp. on prolonged contact | Insoluble |
| 306 | Quinine Salicylate | C,H,O,N | — | — | — | Almost insoluble | 24 |
| 307 | Quinine Sulphate | C,H,O,N, S | — | — | — | 800 | 100 |

| No. | SPECIAL TESTS |
|-----|--|
| 294 | A trace boiled with H_2O gives reddish-violet solution with 1 drop of ferric chloride changing to brown on adding further 2 drops. |
| 295 | Alkaline to litmus, but does not affect phenolphthalein. Forms salts readily; with picric acid forms yellow crystalline picrate. |
| 296 | In presence of alkali it rapidly absorbs oxygen, giving a dark-coloured solution. |
| 297 | Slightly acid solution treated with bromine and then ammonia gives green coloration. |
| 298 | Gives a white ppt. gradually on adding silver nitrate solution to saturated solution. |
| 299 | Dissolves with blue fluorescence in sulphuric acid, acetic acid or tartaric acid. White ppt. with ammonia soluble in excess and in ether. 1 ml. of 1% quinine (dissolved with dilute acid) with 2 to 3 ml. bromine water followed by 1 ml. dilute solution of ammonia gives emerald-green colour (thalleoquin test). Is distinctive for quinine and shows less than 0.0001 g. Belladonna, colchicum, conium, gelsemium, ipecacuanha, opium, nuxvomica do not inhibit the reaction. Use dilute solutions. |
| 300 | <i>See reactions for quinine.</i> Odour of acetic acid is produced on heating with dilute acid. |
| 301 | <i>See reactions for quinine.</i> Aqueous solution is acid to litmus but not to congo red, and is fluorescent. |
| 302 | <i>See reactions for quinine.</i> Aqueous solution is acid to litmus but not to congo red. It fluoresces on the addition of dilute sulphuric acid. |
| 303 | Solutions in dilute acid give reactions of quinine. On warming with sodium hydroxide and iodine solution, iodoform is pptd. |
| 304 | <i>See reactions for quinine.</i> Aqueous solution is neutral or very slightly alkaline to litmus and is not fluorescent. |
| 305 | Bright red powder. |
| 306 | <i>See reactions for quinine.</i> Gives violet coloration with ferric chloride. |
| 307 | <i>See reactions for quinine.</i> |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|--|--|--------------|-------------|------------|---------------------------------|--|
| 308 | Quinoline | C ₈ H ₇ N | — | 236 | 1.095 | Slight | Miscible |
| 309 | Resorcinol | C ₆ H ₂ O | 110-111 | — | — | 0.75 | 1 |
| 310 | Resorcinol Mono- acetate | C ₈ H ₆ O | — | — | — | Slight | Miscible |
| 311 | Rotenone | C ₁₅ H ₁₀ O | 163 | — | — | Insoluble | Soluble (0.124 g. in pure alcohol at 0°) |
| 312 | Saccharin | C ₇ H ₅ O ₂ N, S | About 225 | — | — | 400 | 38 |
| 313 | Saccharin Soluble | C ₇ H ₅ O ₂ N, S ₂ Na | — | — | — | 1.5 at 25° | 50 at 25° |
| 314 | Salacetol (Acetyl- methyl Salicylate) | C ₉ H ₉ O | 67 | — | — | Slight | 14 |
| 315 | Salicin | C ₁₃ H ₁₀ O | 199-201 | — | — | 28 | 80 |
| 316 | Salicyl Salicylate | C ₁₄ H ₁₀ O | 142 | — | — | Almost insoluble | 15 |
| 317 | Salol | C ₁₄ H ₁₀ O | 42-43.5 | — | — | Almost insoluble | 15 |
| 318 | Santonin | C ₁₅ H ₁₀ O | 171-174 | — | — | Almost insoluble | 44 |

| No. | SPECIAL TESTS |
|-----|--|
| 308 | With methyl iodide it reacts to form a methiodide, m.p. 134°. The picrate has m.p. 203° and the hydrochloride 93°. Gives amorphous ppt. with Mayer's reagent which forms yellow needles on adding hydrochloric acid. |
| 309 | On heating 0.05 g. with 0.1 g. tartaric acid and 10 drops conc. sulphuric acid, carmine liquid forms which is yellow on diluting with water. Not pptd. by neutral lead acetate (distinction from catechol). Bluish-violet with ferric chloride changing to yellow on adding ammonia (distinction from catechol and quinol). Resorcinol and phloroglucinol are the only ordinary phenolic compounds giving coloured ppt. immediately in the cold when 2 ml. of 40% formaldehyde and 3 ml. conc. hydrochloric acid are added to a solution of 0.1 g. in 3 ml. 95% alcohol. |
| 310 | Gives reactions of resorcinol and acetic acid. |
| 311 | Forms stable crystalline compounds with carbon tetrachloride (rotenone-carbon tetrachloride), acetone, dichloroacetic acid, etc. From carbon tetrachloride complex pure rotenone is separated by triturating with dehydrated alcohol and then crystallising from hot dehydrated alcohol. Optical rotation of 4% solution in benzene at 20° in 10 cm. tube is -9.04. |
| 312 | Dissolved in 25% potassium hydroxide solution and bromine water added until yellow, bromine substitution body is thrown out. 0.0001 g. heated with 1 mg. resorcinol and 1 drop sulphuric acid gives yellow then dark green. After cooling dissolve in water and add 1 or 2 drops 33% sodium hydroxide solution—intense fluorescence. |
| 313 | Gives reactions of saccharin and leaves residue of sodium sulphate on ignition. |
| 314 | Gives on alkaline hydrolysis reactions of salicylic acid, and the liquid reduces Fehling's, smells of methyl salicylate and burnt sugar, and turns yellow. |
| 315 | Heated with potassium dichromate and dilute sulphuric acid gives salicylic aldehyde (meadowsweet). Dissolves in hydrochloric acid, and on boiling throws out resin (saliretin). Gives blood-red colour with conc. sulphuric acid. |
| 316 | Yields salicylic acid on hydrolysis. |
| 317 | Alcoholic solution ppts. with bromine. Violet with ferric chloride in alcoholic solution. Test for phenol and salicylic acid after heating with alcoholic potassium hydroxide. |
| 318 | Warmed on water-bath with 50% sulphuric acid and a trace of ferric chloride, yellow colour forms, changing through red to violet, or a blood-red can be extracted by amyl alcohol. A crystal warmed with ethyl nitrite solution and a few drops of potassium hydroxide gives a rose-red, with alcoholic potassium hydroxide alone a violet-red colour is obtained. |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|---|-------------------------|-------------|-------------|------------|---------------------------------|---------------------------------------|
| 319 | Scarlet Red | $C_6H_4O_2N$ | 165-185 | — | — | Insoluble | Soluble |
| 320 | Silver Proteinatē | $C_6H_4O_2N$, Ag | — | — | — | Readily | Insoluble |
| 321 | Silver Pro- teinatē, Mild | $C_6H_4O_2N$, Ag | — | — | — | Readily | Insoluble |
| 322 | Sodium Amin- arsonatē | $C_6H_4O_2N$ As, Na | — | — | — | 5 | 125 |
| 323 | Sodium Anti- monyl- tartratē | $C_6H_4O_2Sb$, Na | — | — | — | 1.5 | Insoluble |
| 324 | Sodium Cacodylatē | $C_6H_4O_2As$, Na | About 60 | — | — | 0.5 | 1 |
| 325 | Sodium Glycero- phosphatē | $C_6H_4O_2P$, Na | — | — | — | 4 | Slight |
| 326 | Sodium Lactatē | $C_6H_4O_2Na$ | — | — | — | Soluble | Soluble |
| 327 | Sodium Mandelatē | $C_6H_4O_2Na$ | — | — | — | 1.5 | Almost insoluble |
| 328 | Sodium Phenol- sulphonatē | $C_6H_4O_2S$, Na | — | — | — | 6 | 150 |
| 329 | Sodium Salicylatē | $C_6H_4O_2Na$ | — | — | — | 1 | 6 |
| 330 | Sodium Taurogly- cocholatē | $C_6H_4O_2N$, S, Na | — | — | — | 0.5 | About 2 |
| 331 | Sodium Tet- rabromo- phenol- phthalēin | $C_6H_2O_2Br_4$, Na | — | — | — | Readily | Insoluble |

| No. | SPECIAL TESTS |
|-----|---|
| 319 | Gives a bluish-green solution in sulphuric acid and the solution gives a red ppt. on dilution. |
| 320 | Solution gives no ppt. with ammonium sulphide but becomes black-brown. To 2 ml. 5% aqueous solution add 1 drop of acetic acid; a white ppt. is produced soluble in excess. 10 ml. 1% solution with 5 ml. sodium hydroxide becomes violet in a few minutes on adding 2 ml. 2% copper sulphate solution. |
| 321 | Contains about 20% Ag. |
| 322 | 0.001 g. in 5 ml. water reduces permanganate and gold chloride. Ferrous sulphate solution gives olive-green ppt. Sodium hypobromite gives bluish-red colour. |
| 323 | Responds to tests given for potassium antimonytartrate and also gives sodium flame. |
| 324 | Few drops of aqueous solution with 2 ml. hypophosphorous acid develops garlic odour of cacodyl in a short time. Aqueous solution with mercuric nitrate solution gives white ppt. becoming yellow. |
| 325 | Solid compound is sodium salt of β -glycerophosphoric acid. The salt of the α -acid occurs usually as a 50% solution. An aqueous solution of the former does not reduce cold periodic acid. On incineration pyrophosphate is formed. Lead acetate precipitates but not magnesia mixture. Cold ammonium molybdate precipitates either on standing or heating. |
| 326 | Official substance is viscous liquid containing 68 to 72% of $\text{CH}_3\text{-CHOH-COONa}$. Gives characteristic reactions of lactates. |
| 327 | Gives reactions of sodium and mandelic acid. |
| 328 | Dilute solution does not give yellowish brown with uranium nitrate solution (distinction from salicylate). Incineration gives about 30% Na_2SO_4 . |
| 329 | <i>See</i> salicylic acid. |
| 330 | Taurocholic acid forms shining hygroscopic bitter needles easily soluble in water and alcohol. Solutions dextrorotatory. On heating at 100° or boiling with alkalis or acids, decomposes into cholic acid, $\text{C}_{24}\text{H}_{46}\text{O}_8$, taurine, $\text{C}_2\text{H}_7\text{NO}_2\text{S}$, and glycerin. To an aqueous solution add a crystal of sucrose and conc. sulphuric acid drop by drop; a brownish-red colour is produced changing through violet to blue on keeping (Pettenkofer's bile acid test). |
| 331 | Fused with sodium hydroxide, dissolved in nitric acid and treated with silver nitrate—yellow ppt. of silver bromide. On acidifying aqueous solution light buff ppt. of the phthalein is produced. |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|---------------------------------|------------------------|---|-------------|------------|---------------------------------|---------------------------------------|
| 332 | Sparteine Sulphate | $C_7H_9O_4N_2S$ | 150 when anhyd. | — | — | Less than 0.5 | 5 |
| 333 | Stibophen | $C_7H_5O_3S_2Sb, Na$ | — | — | — | Soluble | Insoluble |
| 334 | Stilbœstrol | $C_{12}H_{10}O$ | 168-171 | — | — | Insoluble | Soluble |
| 335 | Stilbœstrol Dipropionate | $C_{24}H_{20}O_4$ | About 103 | — | — | Insoluble | Soluble |
| 336 | Strophanthin | $C_{12}H_{10}O$ | — | — | — | Soluble | Soluble |
| 337 | Strychnine | $C_{21}H_{22}O_4N_2$ | 270-280 (de- comp.) | — | — | Almost insoluble | 150 |
| 338 | Strychnine Hydrochloride | $C_{21}H_{22}O_4N_2Cl$ | — | — | — | 40 | 80 |
| 339 | Sucrose | $C_{12}H_{22}O_{11}$ | About 160. Does not recryst. on cool- ing. | — | — | 0.5 | About 60 |

| No. | SPECIAL TESTS |
|-----|---|
| 332 | White ppt. with cadmium iodide. Sodium phosphomolybdate gives white ppt. soluble on heating. Ammonium sulphide forms orange colour. To 0.1 g. add 25 ml. ether and a few drops of ammonia followed by a 2% ethereal solution of iodine until the liquid acquires a dark red-brown colour; a greenish-brown crystalline ppt. forms on the sides of the bottle. GRANT'S TEST:—A strip of filter paper moistened with the chloroform extract of the ammoniacal solution of the alkaloid is dried and exposed to bromine then to ammonia fumes. On finally warming, a bright pink colour forms. |
| 333 | The residue from fusion with KOH and KNO ₃ gives reactions for antimony, sodium and sulphates. Solution with ferric chloride gives bluish-green colour turning violet or cherry-red on adding NaHCO ₃ . 1% solution in dilute acetic acid decolorises iodine solution. Solution gives yellow colour with NaOH which disappears on adding acid. |
| 334 | Ultra-violet absorption in dehydrated alcohol at 240 m μ , calculated on dry substance, about 590. |
| 335 | S.V. about 290. Ultra-violet absorption in dehydrated alcohol at 240 m μ , calculated on dry substance, about 390. |
| 336 | Strophanthin, B.P. 1932, is adjusted to standard activity by admixture with lactose. The m.p. and solubility of unadjusted substance vary with the method of extraction. Aqueous solution is dextrorotatory. Dissolved in a cold mixture of sulphuric acid and water (4 : 1), an emerald-green colour is produced (distinction from ouabain). |
| 337 | Trace dissolved in sulphuric acid and a crystal of potassium dichromate moved through solution gives violet colour passing through red to yellow. Vitali's reaction (<i>see</i> Atropine) gives yellow passing to violet. Mandelin's reagent gives violet-blue changing to purple, and becoming red on dilution with water. Phosphomolybdic acid will show 0.0001 g., picric acid 0.00005 g., tannic acid 0.00004 g., mercuric potassium iodide 0.000006 g., potassium bismuth iodide 0.00002 g., platinic chloride only 0.001 g. and gold chloride 0.0001 g. |
| 338 | Gives reactions of strychnine and of hydrochloride. |
| 339 | Becomes brown when heated with conc. potassium hydroxide solution (dextrose becomes brown in the cold). Not directly fermentable—requires inversion by yeast or dilute acids. Does not form osazone or reduce Fehling's until hydrolysed. When a mixture of 1 ml. of saturated nickel ammonium sulphate solution, 1 ml. of sucrose solution and a few drops of sulphuric or hydrochloric acid is boiled, the green colour changes to yellow and then to red. 0.005 g. of sucrose responds, other sugars not interfering. Sucrose can be separated from a dry mixture with dextrose by extraction with hot ethyl acetate, sucrose being insoluble. |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in-) | SOL. IN ALCOHOL 90% (1 in-) |
|-----|--------------------------------|--------------------------|-----------------|-------------|-----------------|--------------------------------|--------------------------------------|
| 340 | Sulphacet- amide | $C_8H_9O_2N,$ S | 181-183 | — | — | 150 | 15 |
| 341 | Sulphanil- amide | $C_6H_7O_2N,$ S | 164.5- 166.5 | — | — | 250 | Slight |
| 342 | Sulpha- pyridine | $C_6H_5O_2N,$ S | 191-193 | — | — | 3000 | 400 |
| 343 | Sulphars- phenamine | $C_6H_5O_2N,$ S,As,Na | — | — | — | Soluble | Insoluble |
| 344 | Sulpha- thiazole | $C_6H_5O_2N,$ S | 202-203 | — | — | 2500 | Slight |
| 345 | Sulphonai | $C_6H_5O_2S$ | 125-127 | — | — | 450 | 80 |
| 346 | Suramin | $C_6H_5O_2N,$ S,Na | — | — | — | Soluble | Soluble |
| 347 | Terpin Hydrate | $C_{15}H_{26}O$ | 116-119 | — | — | 280 | 14 |
| 348 | Terpineol | $C_{15}H_{26}O$ | — | 214- 220 | 0.934- 0.938 | Insoluble | Miscible |
| 349 | Testosterone | $C_{27}H_{48}O$ | 151-156 | — | — | Insoluble | 25 |

| No. | SPECIAL TESTS |
|-----|---|
| 340 | Heated with sulphuric acid and ethyl alcohol, odour of ethyl acetate (distinction from sulphanilamide, sulphapyridine and sulphathiazole). Soluble in NaOH forming soluble sulphacetamide. |
| 341 | An intense violet-colour is produced when heated in a dry tube and ammonia and aniline are evolved. Solution of the diazo-compound gives an orange ppt. with β -naphthol solution. Add platonic chloride in 0.5N hydrochloric acid; warming produces light yellow convex lens-shaped crystal of the chloro-platinate. Then add warm, saturated sodium bromide; orange-yellow hexagonal prisms of the bromo-platinate are produced. Cold saturated picric acid precipitates large yellow needles or prisms. On warming with a neutral solution of picrolonic acid, sheaves or star-shaped clusters of light yellow needles or prisms are produced. |
| 342 | Gives brown colour heated in a dry tube and sulphur dioxide is evolved. Solution of 0.02 g. in 5 ml. of H_2O and 0.25 ml. of N/1 NaOH gives green ppt. with a few drops of copper sulphate. Soluble in NaOH forming soluble sulphapyridine. |
| 343 | Aqueous solution decolorises solution of iodine. 1 ml. of 10% aqueous solution mixed with 5 ml. of dilute hydrochloric acid gives a yellow ppt. in a few minutes and SO_2 is evolved on boiling the mixture but no ppt. is obtained on adding 1.5 ml. of dilute hydrochloric acid to a solution of 0.5 g. in 1.5 ml. of water (distinction from neoarsphenamine). On distilling the aqueous solution acidified with phosphoric acid, the distillate contains formaldehyde. The 10% aqueous solution does not decolorise an equal volume of 0.01% aqueous indigo carmine solution when the mixture is maintained at 50° for 5 minutes (distinction from neoarsphenamine). |
| 344 | Heated in a dry tube it melts, darkens and then evolves ammonia and odour of mercaptan. Solution of 0.02 g. in 5 ml. of H_2O and 0.25 ml. of N/1 NaOH gives greyish purple ppt. with a few drops of copper sulphate. Soluble in NaOH forming soluble sulphathiazole. |
| 345 | Fused with potassium hydroxide it becomes yellow then red, and on diluting with water, blue. On acidifying a transient violet is produced and sulphur pptd. Heated with charcoal, mercaptan is evolved. |
| 346 | Boil for 5 minutes with 50% H_2SO_4 , dilute with 9 volumes of water, cool, add trace of $NaNO_2$, and let stand 1 min., add urea, and after 2 minutes add a few drops of the mixture to a solution of α -naphthylamine buffered with sodium acetate and acetic acid. |
| 347 | On adding a few drops of conc. sulphuric acid to a hot solution the liquid becomes turbid and an odour of lilac is produced. |
| 348 | Strong odour of hyacinth and lilac. |
| 349 | Specific rotation in 1% solution in dioxan, $+104^\circ$ to 112° . Oxime melts at 222° to 223° , semi-carbazone at 225° , benzoate at 194° to 196° , and acetate at 140° to 141° . |

| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|---|----------------|-------------------------|-------------|---------------|---------------------------------|---------------------------------------|
| 350 | Testosterone Propionate | C,H,O | 118-122 | — | — | Insoluble | 30 |
| 351 | Tetrachlor- ethane | C,H,Cl | — | 146 | 1.60 | Insoluble | Miscible |
| 352 | Tetrachlor- ethylene | C,Cl | — | 117- 122 | 1.61- 1.63 | Insoluble | Miscible |
| 353 | Thebaine | C,H,O,N | 193 | — | — | Almost insoluble | Soluble |
| 354 | Theobromine | C,H,O,N | Sub- limes at 290 | — | — | 1000 | 1400 |
| 355 | Theobromine and Calcium Salicylate | C,H,O,N, Ca | — | — | — | Slight | Almost insoluble |
| 356 | Theobromine and Sodium Acetate | C,H,O,N, Na | — | — | — | 2 | 200 |
| 357 | Theobromine and Sodium Salicylate | C,H,O,N, Na | — | — | — | 1 | Insoluble |
| 358 | Theophylline | C,H,O,N | 265-270 | — | — | 160 | 100 |
| 359 | Theophylline and Ethylene- diamine | C,H,O,N | — | — | — | 5 | Insoluble |
| 360 | Theophylline and Sodium Acetate | C,H,O,N, Na | — | — | — | 160 | 100 |
| 361 | Thiosinamine | C,H,N,S | 72-74 | — | — | 17 | 2 |
| 362 | Thiosinamine Ethyl Iodide | C,H,N,I, S | About 70 | — | — | 10 | 1 |
| 363 | Thymol | C,H,O | 48-51 | — | — | About 1000 | 1 |
| 364 | Thymol Iodide | C,H,O,I | — | — | — | Insoluble | Insoluble |

| No. | SPECIAL TESTS |
|-----|--|
| 350 | Specific rotation in 1% solution in dioxan, $+83^{\circ}$ to 90° . Hydrolysed to testosterone and propionic acid with alcoholic KOH, with ppt. of testosterone on dilution with water. |
| 351 | Odour resembles that of chloroform. |
| 352 | Odour resembles that of carbon tetrachloride. |
| 353 | Gives a blood-red coloration with sulphuric acid changing on warming to orange-yellow and eventually to olive green. |
| 354 | Gives murexide reaction (<i>see under Caffeine</i>). Ppts. silver theobromine on adding silver nitrate solution to a very dilute solution acidified with nitric acid. On adding bromine water to the solution in hydrochloric acid, boiling off bromine, and adding a trace of ferrous sulphate and a few drops of ammonia, a purple colour is produced. |
| 355 | Aqueous solution gives violet colour with ferric chloride. Theobromine is pptd. by dilute acids. |
| 356 | Gives murexide reaction. Aqueous solution 1 in 5 neutralised with dilute hydrochloric acid in presence of litmus solution gives white ppt. of theobromine. (A little alkali assists its solubility in water.) |
| 357 | On neutralising aqueous solution with hydrochloric acid, theobromine is pptd. On acidifying with acetic acid and adding ferric chloride, a purple colour is produced. |
| 358 | Gives murexide reaction. Distinguished from theobromine by giving a clear 4% solution in ammonia. |
| 359 | Has a slight ammoniacal odour. Theobromine is pptd. on acidifying the aqueous solution. |
| 360 | Gives reactions of theophylline and also of acetate (after removing the theophylline by neutralising and filtering). |
| 361 | Usually has a faint garlic odour. Heated with lead hydroxide, hydrogen sulphide is removed. |
| 362 | Usually has slight garlic odour. Sodium nitrite and hydrochloric acid give brown ppt. Yellow ppt. with lead acetate insoluble in dilute nitric acid but blackened by the conc. acid. |
| 363 | Characteristic odour. Dissolved in 1 ml. glacial acetic acid and treated with 0.3 ml. sulphuric acid and 1 ml. nitric acid, a bluish-green colour is produced. |
| 364 | Iodine separates on heating with sulphuric acid. |

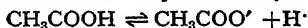
| No. | SUBSTANCE | ELEMENTS | M.P. °C. | B.P. °C. | SP. GR. | SOL. IN WATER (1 in -) | SOL. IN ALCOHOL 90% (1 in -) |
|-----|-------------------------------|------------------------------|-------------|-------------|------------|---------------------------------|---------------------------------------|
| 365 | Thyroxin- sodium | $C_8H_9O_2N$, I, Na | — | — | — | Insoluble | Insoluble |
| 366 | Toluene | C_7H_8 | — | 111 | 0.87 | Insoluble | Miscible |
| 367 | Tribromo- ethyl Alcohol | C_2H_5OBr | 80 | — | — | 33 at 37° | Soluble |
| 368 | Trichlor- ethylene | C_2HCl_3 | — | 88 | 1.47 | Soluble | Miscible |
| 369 | Triethanol- amine | $C_6H_{15}O_3N$ | — | — | 1.12 | Miscible | Miscible |
| 370 | Trinitro- phenol | $C_6H_3O_6N_3$ | 121-123 | — | — | 90 | 10 |
| 371 | Trypar- samide | $C_8H_9O_2N$, As, Na | — | — | — | Readily | Almost insoluble |
| 372 | Tryphan Blue | $C_{12}H_5O_3N$, S, Na | — | — | — | Soluble | Insoluble |
| 373 | Urea | $C_2H_4O_2N_2$ | 130-132 | — | — | 1 | 5 |
| 374 | Urethane | $C_4H_8O_2N_2$ | 47.5-50 | — | — | 2 | 1 |
| 375 | Veratrine | $C_{26}H_{44}O_6N_4$ | 145-155 | — | — | Almost insoluble | 3 |
| 376 | Vinyl Ether | $C_4H_8O(N)$ | — | 28.3 | 0.77 | — | Miscible |
| 377 | Xylene | C_8H_{10} | — | 140 | 0.865 | Insoluble | Miscible |
| 378 | Zinc Phenol- sulphonate | $C_6H_5O_3S$, Zn | — | — | — | 2 | 2.5 |

| No. | SPECIAL TESTS |
|-----|---|
| 365 | When to a solution of a trace in alcohol 50%, prepared with the aid of 1 drop of hydrochloric acid, 1 drop of 20% aqueous sodium nitrite is added, a yellow colour is produced which deepens on boiling; on cooling and adding excess of ammonia the colour changes to red. |
| 366 | By treatment with fuming nitric acid and fuming sulphuric acid at room temperature the 2 : 4-dinitro derivative is obtained, m.p. 70°. |
| 367 | Decomposed on heating into dibromoacetaldehyde and hydrogen bromide. Is supplied as a solution in amylene hydrate. |
| 368 | Colourless, volatile, non-inflammable liquid. |
| 369 | Hydrochloric acid added to the alcoholic solution gives a white ppt. of the hydrochloride which after washing with alcohol and drying has m.p. 173° to 174°. |
| 370 | Gives additional compounds with polynuclear hydrocarbons, e.g. naphthalene picrate (m.p. 150°) is pptd. on cooling 0.1 g. naphthalene and 0.2 g. trinitrophenol in 4 ml. dehydrated alcohol. |
| 371 | Presence of arsenic shown by heating for 45 minutes at about the b.p. with sulphuric and fuming nitric acids, heating again with more nitric acid until no more brown fumes are evolved, then adding solid ammonium sulphate followed by dilute sulphuric acid, boiling with sulphurous acid and testing as usual with hydrogen sulphide. When boiled with sodium hydroxide, ammonia is evolved. Silver nitrate gives a white acicular ppt. |
| 372 | Bluish-grey giving violet colour in water. |
| 373 | Heated above m.p., ammonia is evolved and biuret formed which gives a reddish-violet colour when dissolved in water, made alkaline with sodium hydroxide and treated with 1 drop copper sulphate solution. Urea nitrate pptd. from strong solutions on addition of nitric acid. Decomposed by hypobromite with liberation of nitrogen. |
| 374 | Heated with potassium hydroxide it yields ammonia, potassium carbonate and alcohol. |
| 375 | Mixed with powdered sucrose and sulphuric acid it gives green colour turning blue. Heated with hydrochloric acid on water-bath a blood-red solution is obtained. M.p. is indefinite. |
| 376 | Slightly fluorescent and has characteristic odour. As supplied for anæsthetic purposes contains 3.5% of dehydrated alcohol and 0.01% of phenyl- α -naphthylamine. |
| 377 | A mixture of the <i>o</i> -, <i>m</i> -, and <i>p</i> -compounds, the respective b.p.'s being 142°, 139°, and 138°. Oxidation with alkaline permanganate gives respectively phthalic acid (m.p. 184° decomp.), isophthalic acid (sublimes at 300°), and terephthalic acid (sublimes at 300°). |
| 378 | Yellowish-green ppt. with potassium ferrocyanide insoluble in hydrochloric acid. Decolorises bromine water. |

HYDROGEN ION CONCENTRATION

Many inorganic and some organic substances when dissolved in solvents produce solutions which conduct electricity. The current is carried by ions which are produced by the dissociation of the dissolved substance. Thus a solution of sodium chloride in water contains positively charged sodium ions and negatively charged chlorine ions. Similarly acetic acid solution contains positively charged hydrogen ions and negatively charged acetate ions. The extent to which acetic acid, for example, is dissociated in solution into its ions is regulated by the concentration and the temperature, and on the degree of dissociation will depend the conductivity of the solution.

The equilibrium between undissociated molecule and ions produced by the dissociation is represented by the equation



It is governed by the law of Mass Action, and by applying that law the following relation may be deduced:—

$$\frac{[\text{CH}_3\text{COO}'] \times [\text{H}^{\cdot}]}{[\text{CH}_3\text{COOH}]} = K = \text{Dissociation Constant of the acid,}$$

where the square brackets indicate the concentration of the molecules or ions enclosed by the brackets.

Ordinary tap water, and even ordinary distilled water, will conduct electricity, but as both contain dissolved substances (salts and carbon dioxide in the case of tap water, and carbon dioxide in the case of distilled water) it is not possible to infer from this evidence that water is dissociated into ions. After very careful purification to remove all dissolved gases and other impurities pure water will still conduct electricity to a small extent, and this is due to the dissociation of water into positively charged hydrogen ions and negatively charged hydroxyl ions. The dissociation of water molecules into these ions, as represented by the equilibrium



is also governed by an expression deduced by applying the law of Mass Action:—

$$\frac{[\text{H}^{\cdot}] \times [\text{OH}']}{[\text{H}_2\text{O}]} = K = \text{Dissociation Constant of water.}$$

Since the extent of dissociation of water is small the amount of undissociated water will be very nearly the total amount of water considered and the value of the term $[\text{H}_2\text{O}]$ remains constant for all practical purposes.

$$\text{Hence } [\text{H}^{\cdot}] \times [\text{OH}'] = K \times [\text{H}_2\text{O}] = K_w.$$

K_w has a constant value at constant temperature and is termed

the ionic product of water. Its value is 55 times that of the dissociation constant of water. The ionic product, K_w , represents the extent of dissociation of water at a given temperature, and at 20° it has the value 10^{-14} when the values of $[H^+]$ and $[OH^-]$ are expressed in gram-ions per litre (1 gram-ion of hydroxyl representing 17 g. of hydroxyl ions). On dissociation, pure water will give rise to equal numbers of hydrogen ions, H^+ , and hydroxyl ions, OH^- , and at 20° a litre of pure water will contain 10^{-7} gram-ion of H^+ ($= 0.0000001$) and 10^{-7} gram-ion of OH^- .

If there is added to pure water a substance giving rise to hydrogen ions, say hydrochloric acid, the concentration of H^+ in the solution will momentarily increase to more than 10^{-7} gram-ion per litre. The value of $[H^+] \times [OH^-]$ is restored to its constant value of 10^{-14} by a reduction of $[OH^-]$ effected by combination of some of the OH^- with some of the added H^+ to form undissociated water molecules. The solution now contains more hydrogen ions than hydroxyl ions and is said to be acid in reaction.

Similarly on the addition to pure water of a substance giving rise to OH^- , the concentration of OH^- will momentarily be increased, followed by restoration of the value of $[H^+] \times [OH^-]$ to 10^{-14} by combination of some of the added OH^- with some of the H^+ to form undissociated water molecules. The solution, now containing excess of OH^- over H^+ , is said to be alkaline in reaction. Since the ionic product K_w controls the amounts of H^+ or OH^- in a solution, it is possible to state the extent of acidity, or of alkalinity, by reference only to the hydrogen ion concentration.

Pure water contains 10^{-7} , i.e., 0.0000001 , gram-ion of H^+ per litre; a solution containing more than this, e.g., 0.01 gram-ion of H^+ per litre, is said to be acid, and a solution containing less than this, e.g., 0.000000001 gram-ion of H^+ per litre, is alkaline, and will contain 0.00001 gram-ion of OH^- per litre. Such figures are cumbersome, and a method less liable to error, as suggested by Sorensen, is to use the *logarithm of the reciprocal of the hydrogen ion concentration*, which is referred to as pH .

Pure water containing 0.0000001 gram-ion of H^+ per litre will have

$$pH = \log \frac{1}{0.0000001} = \log \frac{1}{10^{-7}} = \log 10^7 = 7$$

The logarithm reduces the numbers to simpler ones, powers of 10 (the logarithm base), and the reciprocal eliminates the negative sign. Ordinarily pH ranges from about 0 to 14 (more accurately from -0.36 to $+14.5$). pH 0 to 7 is acid and represents solutions containing more H^+ than pure water; pH 7 to 14 is alkaline and represents solutions containing less H^+ and more OH^- than pure water. Since the scale is of logarithmic nature, a change of one pH unit means a tenfold change of H^+ concentration. The amount of additional hydrochloric acid required to change the pH of a solution of hydrochloric acid from 2 to 1 will be ten times that necessary to change the pH from 3 to 2 and a hundred times that

necessary to change the pH from 4 to 3. Such a statement only applies when the solution is free from buffer substances.

The increasing recognition of the importance of control of the acidity or alkalinity of reactions has directed attention to the need for precise methods for the determination of the hydrogen ion concentration of solutions. In addition to considering the changes of hydrogen ion concentration in all branches of volumetric analysis, especially in acidimetry and alkalimetry, attention has been given to the effects of changes of pH in the precipitation of salts and of proteins and other organic substances, in the manufacture of leather, sugar and paper, in brewing, in milk and its preparations, in water purification and sewage disposal, in the study of the fertility of soils, in textiles and dyeing, in biological fluids, in enzyme reactions and in pharmaceutical preparations.

Determination of Hydrogen Ion Concentration

Colorimetric Methods

Theoretical considerations. Indicators are weak acids or bases, or their salts, which undergo partial dissociation in solution, either immediately or after first undergoing a change of molecular structure referred to as a tautomeric change; the ions produced on dissociation differ in colour from the initial undissociated form of the indicator.

The extent to which dissociation occurs is governed by the equilibria $\text{HIn} \rightleftharpoons \text{H}^+ + \text{In}^-$, in the case of an acidic indicator, and $\text{InOH} \rightleftharpoons \text{In}^- + \text{OH}^+$, in the case of a basic indicator.

Considering, then, an acidic indicator, the extent of the dissociation will be governed by the relationship

$$K = \frac{[\text{H}^+][\text{In}^-]}{[\text{HIn}]}$$

where K is the dissociation constant of the indicator and has a constant value at a given temperature. If the indicator is added to water not only must the dissociation of the indicator be such that the above relation holds but also such that

$$[\text{H}^+] \times [\text{OH}^-] = 10^{-14}.$$

If an alkaline substance such as NaOH is now added, H^+ will be withdrawn from solution by combining with some of the added OH^- until the value of K_w is restored to 10^{-14} . The withdrawal of hydrogen ions causes a disturbance of the equilibrium governing the dissociation of the indicator and more indicator molecules must dissociate to produce ions. From similar considerations it will be seen that the addition of acid would decrease the dissociation of the indicator.

$$\text{Since } K = \frac{[\text{H}^+] \times [\text{In}^-]}{[\text{HIn}]}$$

$$\text{it follows that } [\text{H}^+] = K \times \frac{[\text{HIn}]}{[\text{In}^-]}$$

$$\text{or that } \text{pH} = \log_{10} \frac{1}{[\text{H}^+]} = \log_{10} \frac{1}{K} + \log_{10} \frac{[\text{In}^-]}{[\text{HIn}]}.$$

At any given temperature the value of K is constant so that at that temperature

$$\text{pH} = \text{Constant} + \log_{10} \frac{[\text{In}^-]}{[\text{HIn}]}.$$

The colour given by the indicator will depend on the actual concentrations of indicator ions, In^- , and of undissociated indicator molecules, HIn . It is found, in general, that the colour due to the indicator ions is not visible until 10% of the indicator has become ionised, due to added alkali in the case considered, and that the colour of the undissociated indicator does not become completely masked to the eye until the indicator has undergone 90% ionisation or salt formation. From these facts the transition interval from first colour change to final colour can be calculated in terms of pH units:—

$$\begin{aligned}\text{Initial pH} &= \log_{10} \frac{1}{K} + \log_{10} \frac{10}{90} \\ &= \log_{10} \frac{1}{K} - 1 \text{ (approx.)}\end{aligned}$$

$$\begin{aligned}\text{Final pH} &= \log_{10} \frac{1}{K} + \log_{10} \frac{90}{10} \\ &= \log_{10} \frac{1}{K} + 1 \text{ (approx.)}\end{aligned}$$

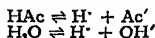
$$\begin{aligned}\therefore \text{Interval of colour change} &= \text{Final pH} - \text{Initial pH} \\ &= 2 \text{ pH units (approx.)}\end{aligned}$$

and the colour changes at $\text{pH} = \log_{10} \frac{1}{K} \pm 1$.

It follows that the actual pH at which the indicator functions depends on the value of K , the dissociation constant of the indicator, for when the indicator colour has undergone half-change $\text{pH} = \log_{10} \frac{1}{K}$.

The choice of indicator for acid-alkali titrations is determined by the products of the reaction and is such that the indicator changes colour at the pH value of the reaction products. On adding a suitable indicator to a solution the colour obtained is determined by the pH of that solution. Comparison of that colour with the colour produced in standard solutions of known pH value by the same amount of indicator forms the basis of the colorimetric determination of pH values of solutions.

The standard solutions used are not liable to changes of pH due to carbon dioxide or alkali from the glass of the vessels, and are known as *buffer solutions*. Dilute solutions of strong acids and strong bases are extremely sensitive to carbon dioxide and alkali from glass and are useless as standards. The buffer solutions contain a salt of one of the weaker acids together with an acid or a base. Consider a solution containing sodium acetate, a salt which is practically completely dissociated in water, together with acetic acid. The following equilibria are obtained:—



The total acetate ions in the solution are those from sodium acetate and those from acetic acid and the total hydrogen ions are those from the acid and those from water. The equilibria are governed by the relations:—

$$\frac{[\text{H}^+][\text{Ac}^-]}{[\text{HAc}]} = K_A, \text{ and } [\text{H}^+][\text{OH}^-] = K_W.$$

If to such a solution small amounts of an acid be added, the hydrogen ions added would combine with some of the excess of acetate ions, the value of K_A being maintained and the original hydrogen ion concentration of the solutions. Larger amounts of added acid would produce small changes only in pH . On addition of small amounts of alkali, hydrogen ions would combine with the added hydroxyl ions forming undissociated water molecules, and the value of K_A would be restored by further dissociation of acetic acid molecules, the pH value of the solution thus remaining almost unchanged.

A Universal Indicator and Buffer Solution.

When the approximate pH of the solution only is required this can rapidly be ascertained by the use of a mixed or universal indicator, which gives a series of colours at different values of pH. A universal indicator can be prepared by dissolving methyl orange 0.04 g., methyl red 0.02 g., naphtholphthalein 0.18 g., and phenolphthalein 0.08 g. in 100 ml. of 70% alcohol, and this gives the whole range of spectrum colours in the correct order from red (pH 3) to violet (pH 11). The hydron concentration of a solution is found by adding 5 drops of this indicator to 10 ml. and from the colour obtained the value of the pH can be read off from the table below, but it is better to compare the tint with that given by solutions of known pH. For such solutions it is convenient to use the Universal Buffer Mixture proposed by E. B. R. Prideaux and A. T. Ward (*J. chem. Soc.*, 1924, 426), which gives solutions of definite pH from 2 to 12 by neutralising with caustic soda solution. This solution contains H_3PO_4 1.961 g., phenylacetic acid 2.722 g., and boric acid 1.238 g. in 500 ml., and for use 10 ml. is mixed with the requisite amount of N/5 NaOH and made up to 20 ml.

The volume of N/5 NaOH required for some pH values is shown in the table below; for intermediate values the graph in the original paper (*loc. cit.*) must be consulted. The formula $\text{pH} = 0.773 + 1.185 V$, where $V = \text{ml. of N/5 NaOH per 10 ml. of solution}$, is sufficiently accurate for many purposes, and holds between $V = 1.5 \text{ ml.}$ and $V = 9 \text{ ml.}$ The colour changes of the universal indicator and the composition of the universal buffer solution with different values of hydron concentration are also shown:—

| Approx. value of pH | Ml. N/5 NaOH per 10 ml. Buffer Solution in 20 ml. | Colour with Universal Indicator (5 drops per 10 ml. solution) | Approx. value of pH | Ml. N/5 NaOH per 10 ml. Buffer Solution in 20 ml. | Colour with Universal Indicator (5 drops per 10 ml. solution) |
|---------------------------|--|---|---------------------------|--|---|
| 2.0 | 0.0 | — | 7.5 | 5.6 | yellowish-green |
| 3.0 | 2.0 | crimson | 8.0 | 6.0 | green |
| 4.0 | 2.8 | red | 8.5 | 6.2 | bluish-green |
| 5.0 | 3.6 | orange-red | 9.0 | 7 | greenish-blue |
| 5.5 | 4.0 | orange | 9.5 | 7.3 | blue |
| 6.0 | 4.5 | orange-yellow | 10.0 | 7.7 | violet |
| 6.5 | 4.8 | yellow | 11.0 | 8.5 | reddish-violet |
| 7.0 | 5.2 | greenish-yellow | 12.0 | 10.0 | — |

More Accurate Colorimetric Determination.

Having determined the approximate pH of the solution by a universal indicator, an indicator of suitable pH range is selected (see list of indicators, p. 604).

For colourless solutions: To 10 ml. of the solution in a hard glass test-tube add 0.5 ml. of indicator solution and mix. Into a series of test-tubes similar to the one used above introduce 10 ml. of the buffer solutions of the pH range required, to each add 0.5 ml. of indicator solution and mix. The buffer solution whose colour most nearly matches that given by the solution tested indicates the pH value of the latter.

For turbid or slightly coloured solutions: In front of the tube containing the solution under test and indicator, place a tube containing the same volume of distilled water as of solution used; in front of the tube containing the buffer solution and indicator place a tube containing the same volume of solution being tested, examine the colours by looking through the pairs of tubes. When only small quantities of liquid are available capillary tubes may be used in place of test-tubes. A scratch is made about 1 inch from an end of a capillary tube about 4 inches long, to the other end of which a small teat is fitted. The tube is filled to the scratch with solution, a bubble of air introduced, then filled to the scratch with indicator solution and the solutions mixed by emptying the tube on to a small watch glass and refilling with the mixed solutions. The same procedure is followed using buffer solutions of known pH value and indicator, tubes of similar size and bore being used. The colours obtained are compared as before.

The Bicolorimeter: In order to avoid the numerous standards required in the methods described, it is possible to match all the shades of colour in a given indicator from the acid to the alkaline side by use of two movable wedges, one filled with an acid solution of the indicator and the other with an alkaline solution, thus reducing the number of standards required with a single indicator to two. By altering the positions of the two wedges different proportions of the two colours may be superimposed and viewed side by side with the solution of unknown pH containing the indicator. The Bicolorimeter (Myers, *J. biol. Chem.*, 1922, 54, 675, and Hellidge, *J. sci. Instrum.*, 1927, 4, 327) contains three wedges sliding up and down in a closed box. The third wedge is used to compensate, if required, for turbidity or colour in the sample. Readings are taken from adjustable scales which emerge from the top of the instrument. Light passes horizontally through the wedges and the cell containing the solution tested, and by an arrangement of prisms the two fields are viewed side by side through a magnifying eye-piece. The bicolorimeter has found extensive application in microcolorimetric methods for the determination of the pH of blood and urine (Myers, Schmitz and Booker, *J. biol. Chem.*, 1923, 57, 209; Myers and Muntwyler, *ibid.*, 1928, 78, 225 and 243).

Preparation of Buffer Solutions.

Details are given in Appendix III of the B.P. 1932 for the preparation of solutions of standard pH, based on the work of W. M. Clark and H. A. Lubs (*J. Bacteriol.*, 1917, 2, 1, 109, 191).

When buffer solutions are required having pH 1.4 to 6.2 the use of carbonate-free caustic soda can be avoided by using the solutions described by W. L. German and A. I. Vogel (*Analyst*, 1937, 271).

For the preparation of buffer solutions of pH 4.0 to 8.4 one standard solution only and carbonate-free caustic soda are used in the method due to H. T. S. Britton and R. A. Robinson (*J. chem. Soc.*, 1931, 1456). The standard solution required contains citric acid, 6.002 g.; boric acid, 1.767 g.; barbitone, 5.260 g.; the dissolved substances being made up to 1 litre with freshly boiled and cooled distilled water. The following table gives the amounts of 0.2N sodium hydroxide solution to be added to 100 ml. of the standard solution above, the mixture then being diluted to 200 ml.

| | | | | | | |
|---------------------|-------|-------|-------|-------|-------|-------|
| pH | 4.0 | 4.20 | 4.40 | 4.60 | 4.80 | 5.00 |
| Volume of 0.2N NaOH | 1.22 | 3.57 | 5.90 | 8.25 | 10.60 | 12.95 |
| pH | 5.20 | 5.40 | 5.60 | 5.80 | 6.00 | 6.20 |
| Volume of 0.2N NaOH | 15.30 | 17.63 | 20.00 | 22.33 | 24.67 | 27.00 |
| pH | 6.40 | 6.60 | 6.80 | 7.00 | 7.20 | 7.40 |
| Volume of 0.2N NaOH | 29.35 | 31.70 | 34.05 | 36.40 | 38.75 | 41.10 |
| pH | 7.60 | 7.80 | 8.00 | 8.20 | 8.40 | — |
| Volume of 0.2N NaOH | 43.43 | 45.77 | 48.13 | 50.47 | 52.80 | — |

For intermediate values of pH the volume (x) ml. of 0.2N NaOH required may be calculated from the formula

$$\text{pH} = 3.896 + 0.0853 x$$

Sodium Hydroxide for Buffer Solutions.

The sodium hydroxide solution used should be as free as possible from carbonate. For ordinary purposes in which absolute freedom from carbonate is not necessary the following method, adopted by Sorensen, may be used:—100 g. of a good sample of sodium hydroxide is dissolved in 120 ml. of boiled and cooled distilled water. The solution is placed in a tall stoppered cylinder, and the impurities, mainly carbonate, allowed to settle out by leaving the cylinder standing overnight. After decantation of the clear liquid and filtration through glass wool, the solution, which is about 17N, is diluted to about N/1 concentration with freshly boiled and cooled water, standardised against hydrochloric acid or recrystallised benzoic acid. N/5 solution is then prepared by diluting the standardised alkali with freshly boiled and cooled water.

For more accurate work the alkali should be prepared from electrolytic sodium amalgam. Redistilled mercury is placed in a separating funnel, above which is a saturated solution of pure sodium chloride. Electrolysis is effected by means of three 2-volt accumulators placed in series. The mercury is made the cathode by connecting the negative pole to it by a wire dipping into a mercury contact placed in a closed glass tube, passing through the end of which is a platinum wire whose other end is completely immersed in the mercury, and the anode is a platinum wire dipping into the sodium chloride solution. As the electrolysis proceeds shaking may be necessary to prevent the crystallisation of the amalgam. When sufficient amalgam is formed, the current is stopped and the amalgam cautiously dropped into freshly boiled and cooled water, the end of the tube of the separating funnel being below the level of the water.

Errors in Colorimetric Methods.

Apart from ordinary manipulative errors, there are a number of errors inherent in the various indicators themselves.

Acid Error. As indicators function either as weak acids or bases, it is apparent that in the minute concentrations used in test solutions, they can have no appreciable effect upon the pH of well buffered solutions. If the solution, however, is unbuffered the indicator itself may affect the pH of the solution and introduce a difference between observed and actual values. Thus a solution of sodium hydroxide of pH 10 appears to have a pH of 8 to 9 when examined with phenolphthalein. The existence of such an error is revealed by using other indicators with different dissociation constants.

"Salt Error." The presence of neutral salts causes a variation in the colour changes of indicators and also in the pH values at which these changes normally occur. The salt influences the ionic equilibria involved in the functioning of an indicator and also may have some effect on the optical absorption of the two forms of the indicator.

The "Protein Error." Proteins and their decomposition products are colloidal and amphoteric. They may interact with acidic and basic indicators; in consequence the indicators may become partly adsorbed and the colours affected. Colorimetric methods for pH determination in solutions of proteins are of doubtful value and electrometric methods should be employed. (See McCrumb and Kenny, *J. Soc. chem. Ind., Lond.*, 1930, 49, 428T.)

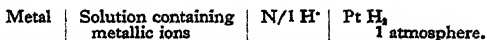
The Temperature Effect. An increase of temperature has a marked effect on electrolytic dissociation, water, acids and bases, and hence indicators, ionising to a greater extent. The principal effects of increasing temperature are to diminish the pH scale, to reduce the pH corresponding to neutrality and to reduce the limiting pH values of the various indicator transition ranges. The pH value of neutrality is 7 at 18°; 6.4 at 70°; and 6.1 at 100°. The errors incurred by the small variations in laboratory temperatures, e.g., 15° to 25°, do not normally amount to more than the errors of the colorimetric methods used.

Effect of Alcohol. The presence of alcohol in aqueous solutions usually diminishes the extent to which indicators are ionised and hence the sensitivity of the indicator. A solution containing phenolphthalein just reddened by alkali becomes gradually paler in colour and finally colourless on the continued addition of alcohol. Thymolphthalein, phenolphthalein, thymol blue (alkaline range), tropaeolin 0, tropaeolin 00, methyl orange and methyl yellow do not undergo a change in colour until considerably more reagent has been added in aqueous alcoholic than in aqueous solution. Bromophenol blue and thymol blue (acid range) become more susceptible to colour change in the presence of alcohol.

Electrometric Methods.

In view of the errors to which colorimetric methods for pH determinations are liable, electrometric methods are to be preferred for accurate determinations with colourless solutions and are necessary with coloured solutions. The following are the principles involved in the use of electrometric methods:—

When a metal is dipped into a solution containing its ions, one of two processes normally occurs—either the ions tend to take up electrons from the metal, become neutral atoms and deposit on the metal, or the metal atoms tend to lose electrons, become positively charged ions and pass into the solution. In the former case the metal acquires a positive potential with respect to the solution, in the latter case a negative potential. The potential acquired may be measured by making the metal and the solution of its ions one electrode of a cell the other electrode of which consists of a solution of normal hydrogen ion concentration into which dips a platinum electrode coated with platinum black and around which is bubbled pure hydrogen gas at one atmosphere pressure. The arrangement of such a cell is represented diagrammatically:—



The processes which may occur at the hydrogen electrode are the same as those described for the metal in a solution of its ions. The hydrogen is adsorbed by

the platinum black, which thus functions as a hydrogen "metal" electrode, which may acquire a positive or negative potential as before. The electromotive force of the cell set up will be the algebraic difference of the potentials of the electrodes. Owing to the uncertainty which still exists as to the absolute potential of a hydrogen electrode, the "normal" hydrogen electrode (prepared as described) is taken as arbitrary zero of electrode potentials. On this scale, therefore, the measured E.M.F. will indicate the potential of the metal electrode in the solution of its own ions.

If a cell is set up consisting of two electrodes, one being the "normal" hydrogen electrode described and the other platinum coated with platinum black, dipping into a solution of unknown hydrogen ion concentration, pure hydrogen gas at one atmosphere pressure being bubbled around the platinum, then the E.M.F. at 18° is given by the formulæ:—

$$E = 0.058 \times \text{pH of solution tested, and at other temperatures (T being the temperature on the absolute scale)}$$

$$E = 0.0001982 \times T \times \text{pH of solution tested. Hence from the measured value of the E.M.F. the pH of solutions can readily be calculated.}$$

The preparation of a normal hydrogen electrode for use as a standard is a difficult task and strictly reproducible potentials are not always obtained even by experienced workers. For this reason secondary standard electrodes are preferred whose potentials are known on the so-called "normal hydrogen" scale in which the potential of the normal hydrogen electrode described, is arbitrarily taken as zero. The calomel electrodes are most used. In these a layer of mercury in a suitable vessel is covered with a thin layer of electrolytically prepared calomel and a solution of potassium chloride saturated with calomel. A platinum wire is sealed into the container such that contact is made with the layer of mercury. According to the strength of the potassium chloride solution, the potential of the electrode varies and the table gives corresponding values:—

| Strength of solution of KCl saturated with calomel .. | N/10 | N/1 | 3.5N | Saturated |
|---|--------|--------|--------|-----------|
| Potential of electrode in volts at 18° | 0.3370 | 0.2864 | 0.2549 | 0.2504 |

The pH of a solution can readily be determined by setting up a cell, the positive electrode of which is a calomel electrode and the negative electrode consists as before of platinised platinum dipping into the solution of unknown pH, hydrogen gas being bubbled around the platinum at one atmosphere pressure. The E.M.F. of the cell is determined experimentally as described later and the pH at 18° calculated from the expression

$$\text{pH} = \frac{(\text{Measured E.M.F.}) - (\text{Potential of calomel electrode})}{0.058}$$

At other temperatures the value is given by

$$\text{pH} = \frac{(\text{Measured E.M.F.}) - (\text{Potential of calomel electrode})}{0.0001982 T}$$

where T is the temperature on the absolute scale.

Quinhydrone Electrode. The use of gaseous hydrogen can be avoided by adding to the solutions containing hydrogen ions quinhydrone, a compound of one molecule of hydroquinone, $\text{C}_6\text{H}_4(\text{OH})_2$, with one molecule of quinone, $\text{C}_6\text{H}_4\text{O}_2$. A platinum electrode dipping into this solution acquires a potential whose value is dependent on the hydrogen ion concentration of the solution and is given by the formula:—

$$\begin{aligned} E_Q &= 0.704 + 0.058 \log_{10} \text{H}^+ \text{ volts at } 18^\circ \\ &= 0.704 + 0.0001982 T \log_{10} \text{H}^+ \text{ at } T^\circ \text{ absolute.} \end{aligned}$$

Such an electrode is referred to as a "quinhydrone" electrode.

The chief advantages of the quinhydrone electrode are that it attains equilibrium rapidly and that it may often be used in solutions containing oxidising agents and salts of metals more noble than hydrogen, for which the hydrogen electrode is inapplicable. Its disadvantages are that it cannot be used in solutions of pH greater than 8, that appreciable errors occur in the presence of protein and that occasionally the presence of salts causes small errors. From the measured value of the E.M.F. of a cell, of which one electrode consists of polished platinum dipping into the solution under test and containing quinhydrone and the other is a standard calomel electrode, the pH of the solution tested can be calculated using the formula:—

$$\text{pH} = \frac{0.704 - (\text{Potential of calomel electrode}) - (\text{Observed E.M.F.})}{0.0001982 \times \text{Absolute Temperature}}$$

Glass Electrode. The time taken for a hydrogen electrode to reach equilibrium, its inapplicability to solutions containing oxidising agents, and its susceptibility to platinum "poisons" are factors which limit its usefulness. Equally limited is the use of a quinhydrone electrode as described in alkaline solution, in the presence of proteins and in the presence of certain salts. For these reasons the so-called "glass electrode" is sometimes preferred. A very thin-walled bulb is blown in the end of a glass tube made of high conductivity glass, this is filled with N/1 hydrochloric acid saturated with quinhydrone, a platinum wire dipping into this solution. The glass electrode so prepared is dipped into the solution of unknown pH, a calomel electrode also dipping into the solution. If there exists a different concentration of hydrogen ion in the solution from that of N/1 HCl, then a difference of potential occurs between them urging the movement of hydrogen ions through the thin glass membrane in one direction, which determines the potential acquired by the platinum wire. The electrode is calibrated by using as the solution outside the membrane buffer solutions of known pH. The E.M.F. of the cell shown diagrammatically is determined:—

| | | | |
|---|-------------------|--------------------|----------------------|
| N/1 HCl Pt saturated with quinhydrone | Glass membrane | Buffer solution | Calomel electrode |
|---|-------------------|--------------------|----------------------|

Having measured the E.M.F. of the cell using the standard buffer solutions, a graph is prepared in which pH values are plotted against E.M.F. The relationship is linear up to pH 10. The pH value of an unknown solution can then be determined by placing it in the vessel outside the glass membrane and measuring the E.M.F. The pH value can be read off from the calibration curve.

Convenient glass electrodes are described by Morton (*J. Sci. Instrum.*, 1930, 187), Hughes (*J. Amer. chem. Soc.*, 1922, 2860) and Kerridge (*J. Sci. Instrum.*, 1926, 404), the latter being especially useful for work with biological fluids as only small quantities (as little as 0.5 ml.) of solution are required. The glass electrode cannot be employed, as calibrated, for strongly alkaline solutions (about pH 10) though a calibration method suggested by Powney and Jordan enables this difficulty to be overcome (see *J. Soc. chem. Ind., Lond.*, 1937, 133T). Glass electrodes are liable to error due to a protein film when used for measurement of pH values of protein solutions. The need for thorough cleaning by chromic acid is emphasised by G. E. Shaw (*Quart. J. Pharm.*, 1940, 271).

Measurement of Electromotive Force of Experimental Cells.

In setting up the experimental cells, the electrodes are connected by means of a bridge vessel containing potassium chloride solution or an agar gel containing potassium chloride. By using such a bridge the so-called "liquid junction potentials" which arise from diffusion of the electrode solutions into one another are almost eliminated. The E.M.F. of the experimental cell is determined by means of a potentiometer and the fundamental principles of the Poggendorf Compensation method are applied. An accumulator is connected to the ends of a uniform wire AB. With the standard cell in circuit the length of wire, AC₁, is found by means of a sliding contact C such that no current flows through the sensitive galvanometer or capillary electrometer indicating that the fall of potential from A to C is equal to the E.M.F. of the standard cell. By means of a two-way switch the experimental cell is put in circuit in place of the standard

cell and the length of wire, AC_2 , is determined for which no current flows through the galvanometer. Then

$$\frac{\text{E.M.F. of standard cell}}{\text{E.M.F. of experimental cell}} = \frac{AC_1}{AC_2}$$

The Weston cell most commonly used as standard has an E.M.F. of 1.0183 volts and hence the E.M.F. of the experimental cell will be $\frac{AC_1}{AC_2} \times 1.0183$ volts.

In measuring the E.M.F. of these experimental cells it is necessary to avoid taking any appreciable current from them to avoid disturbing the equilibrium between electrode and solution. Hence the sliding contact should only make contact with the potentiometer wire for very short periods of time. This difficulty may also be overcome by using the thermionic valve.

Some Applications of pH Measurement and Control.

Electrometric Titrations. It can be shown, both theoretically and practically, that during the neutralisation of an acid by a base there is generally a sudden change of pH in the vicinity of the equivalence point. If, therefore, a hydrogen electrode be inserted in a solution of an acid, and an alkali added gradually there will be a sudden change in the potential of the electrode as the equivalence point is attained. In general, the slope of the curve in which the pH or electrode potential is plotted against the amount of titrant added is a maximum at the end-point. To determine the latter accurately the quantity $\Delta E/\Delta v$ should be plotted against the volume of titrant added, ΔE being the change of potential resulting from the addition of a definite small increment Δv , e.g., one drop, of titrant. The amount of alkali corresponding to the point at which $\Delta E/\Delta v$ is a maximum represents the equivalent of the quantity of acid being titrated.

In one form or another the hydrogen electrode has been used in a very large number of electrometric titrations of analytical importance. The variety and scope of these is to be seen from the following examples: neutralisation of inorganic, organic and amino-acids in coloured and colourless solutions; analysis of mixtures of strong and weak acids; titration of salts of weak acids by strong acids; of magnesium and aluminium salts with alkali; of boric acid alone and in the presence of polyhydroxy compounds; determination of the acidity of galenical preparations, of fruit juices, beer, milk, biological fluids, soil extracts, tannery liquids, fats, dyestuffs, insulating and petroleum oils. The particular type of hydrogen electrode used depends on the nature of the solution being titrated and on the degree of accuracy desired, as well as on general convenience.

By following the neutralisation of hypophosphorous acid with a glass electrode Morton showed that phosphorous and phosphoric acids are included when titration of the acid is carried out by the B.P. '32 method, using sodium hydroxide with methyl orange as indicator (*Quart. J. Pharm.*, 1930, 438).

Glass electrode study of the titration with alkali of citric acid saturated with ferric hydroxide led to the conclusion that some combination between iron and citric acid occurs in scale preparations.—C. Morton, *Quart. J. Pharm.*, 1931, 161.

The changes occurring in various chemical substances on sterilisation by steaming was followed by colorimetric determination of pH.—H. Davis, *Quart. J. Pharm.*, 1934, 379.

The study of the influence of pH on the racemisation of *l*-adrenaline shows that it is rapid in hydrochloric acid solutions of pH 0.1 but at higher pH values, 1.4 to 3.7, it becomes negligibly small.—L. Haddock, *Quart. J. Pharm.*, 1933, 496.

Investigation of the influence of pH on the stability of hypochlorite solutions indicates that a more desirable preparation for therapeutic use is obtained when the pH is lower than 9.7, the value obtained using Dakin's formula. The preparation of the B.P. '32 has pH 9 to 9.5.—H. Davis, *Quart. J. Pharm.*, 1931, 360.

Electrometric determination of pH has been used to follow the changes occurring during storage of sterilised solutions of quinine dihydrochloride.—F. F. Johnson, *J. Amer. pharm. Ass.*, 1937, 26, 1227.

The toxicity of strychnine solutions varies with the pH of the solutions.—J. Travell, *J. Pharmacol.*, 1940, 69, 21.

Studies of the value of sodium metabisulphite, cysteine, phenylhydrazine and protocatechuic aldehyde at various pH values on stabilising adrenaline in solutions of procaine and adrenaline showed that sodium metabisulphite was the most suitable but that the amount necessary varied with the pH of the solution.—G. Woolfe, *Quart. J. Pharm.*, 1941, 56.

Studies of the rate of decomposition of procaine in acid solutions showed that the pH of maximum stability for procaine was 3.3.—K. Bullock and J. S. Cannell, *Quart. J. Pharm.*, 1941, 241.

The rate of hydrolysis of amylocaine has been determined at various pH values.—K. Bullock and J. S. Cannell, *Quart. J. Pharm.*, 1941, 313.

A similar study has been made of the rate of decomposition of amethocaine at various pH values.—K. Bullock and J. S. Cannell, *Quart. J. Pharm.*, 1941, 323.

Solutions of lobeline hydrochloride may be sterilised and stored without any appreciable decomposition occurring provided a slight excess of N/1000 hydrochloric acid is added to the solution, as shown by correlation of pH of solution with decomposition.—F. Reimers, per *Quart. J. Pharm.*, 1938, 148.

Studies of the effect of pH of a solution of senega saponin on its stability showed that at pH 9 to 11 the solution was much less stable on storage as shown by hæmolytic index than at pH 3 to 7.—S. A. Schou and C. J. T. Madsen, per *Quart. J. Pharm.*, 1938, 125.

In processes employing peroxides such as sodium peroxide, hydrogen peroxide and sodium borate the determination and control of pH are important. Accurate determinations are possible with the glass electrode, corrections only being necessary above pH 10 or with high concentrations of sodium ion. Curves are given relating pH values with peroxide concentration in the presence of acid or alkali and are of value in preparing peroxide solutions of definite pH.—J. S. Reichert and H. G. Hull, *Industr. Engng Chem. (anal. Edn.)*, 1939, 11, 311.

For notes on the importance of pH in sterilisation, see p. 1002.

Blood. Constancy of pH of blood, see p. 678.

For determining the pH of the blood *in vitro* any of the methods described above may be used. For whole blood the electrometric method is exclusively available. Care must be taken that none of the CO₂ is lost by volatilisation. The pH of streaming blood may be determined by inserting a platinum wire, which has been coated electrolytically with manganese dioxide, into the blood stream. Such a wire functions as a hydrogen electrode and can be made part of a cell (Gesell and Hertzmann, *Proc. Soc. exp. Biol., N.Y.*, 1925, 30, 298).

For measuring pH in subcutaneous tissue a regular hydrogen electrode (using hydrogen gas) has been adapted by perforating the skin and introducing a glass capillary carrying a platinum wire. Hydrogen gas and carbon dioxide (at 40 mm. pressure) are conducted through the tube. The pH of the tissue is found to be 7.09 to 7.29, which is slightly more acid than blood. Following exhaustion by muscular exercise the pH drops to about 6.6.—Schade, Neukirch and Halpeut, *Z. ges. exp. Med.*, 1921, 24, 11.

Enzymes. The stability and activity of enzymes is appreciably influenced by the pH of the media in which the enzymes are used and the adjustment of solutions to definite pH is of the utmost importance for optimum activity. Similarly, the adjustment of pH may be necessary to prevent reactions which enzymes would otherwise induce. For example, in the B.P., 1932, identity tests for pancreatin and pepsin, the activity of these enzymes is destroyed by adjusting to a particular pH. A complete table of the pH ranges of activity and

stability of enzymes is given in *Enzymes*, by Waksman and Davison (Williams and Wilkins, 1926), or *Enzymes*, by Haldane (Longmans, Green and Co., 1930).

Iso-electric Point. Certain substances such as amino-acids, proteins and aluminium hydroxide pass either into true solutions or colloidal solutions and react either as bases or as acids. Such solutions are referred to as ampholytes. When the hydrogen ion concentration of the solution is such that the activity of the ampholyte as a base is equal to its activity as an acid, the ampholyte is at its minimum chemical activity and exists in its maximum undissociated condition. When this obtains the ampholyte is at its iso-electric point and acquires its minimum solubility. The adjustment of pH of solutions to the iso-electric point is of importance in the precipitation from solutions of proteins and related substances. The isolation of hormones such as thyroxine, insulin, gonadotrophins, prolactin (lactogenic hormone), adrenotrophic and thyrotrophic hormones depends on the pH of the mother liquors. Insulin is soluble in water and in alcohol up to 80% provided the solution is not at the iso-electric point of insulin (pH 5.0). Proteins in milk are stated to be determined to within 0.1% by the following method:—Run from a burette small amounts of N/10 hydrochloric acid into a measured amount of milk and determine the pH. Take three or four readings and construct a curve of pH against acid added. As the curve is practically linear, it is possible to interpolate the amount of N/10 hydrochloric acid to be added to take the pH of milk from one value to another. Such an amount of acid will be proportional to the protein content. By using 10 ml. of milk the amount of N/10 hydrochloric acid necessary to change the pH from 6.65 to 5.2 may be taken as numerically equal to the percentage of protein in the milk.—Harris, *Proc. Roy. Soc. Ser. B.*, 1924, 97, 372, see also Clark, *J. Dairy Sci.*, 1927, 199.

A simple procedure has been devised for the rapid approximate determination of the isoelectric point of soluble proteins. The method depends upon the precipitation of proteins in their anionic form by cationic detergents ("invert soaps"). This precipitation should commence at the isoelectric point. A series of buffer solutions is prepared at intervals of 0.2 on the pH scale, and to 2 ml. of each is added 5 drops of a 0.1% solution of a suitable cationic wetting agent, such as a mixture of higher alkyl dimethylbenzylammonium chlorides (Zephiral), together with sufficient aqueous solution of protein to give a final concentration of about 10 mg. per ml. The pH of the most acid mixture to yield a precipitate is indicated as the isoelectric point.—W. G. Jaffe, *J. biol. Chem.*, 1943, 148, 185.

The colour intensity of dextrose solutions, which has been observed with a Zeiss Pulfrich photometer, is independent of pH between the range pH1 to pH5, but between the values 5.5 to 6.2 there is a rapid change to a darker brown. The colour of heated dextrose solutions varies with pH, the minimum value being reached in the range 2.3 to 3.0. The pH of dextrose solutions should be maintained within these limits during manufacturing and processing.—W. Kroener and K. Kothe, *Industr. Engng Chem.*, 1939, 31, 248.

The influence of pH change on the fractionation of protein and antitetanus serum.—A. V. Markovich and I. M. Khaustova, *Chem. Abstr. B.*, 1939, 6893.

The precipitation of hydroxides, basic salts, phosphates, sulphides and silicates is materially affected by the pH of the solution and a full account of the study of pH influence is given in *Hydrogen Ions*, by H. T. S. Britton (Chapman and Hall, 1942). The influence of small changes of pH value on the adsorption of other metallic ions during precipitation of iron, chromium or aluminium as hydroxides is discussed by G. J. Austin (*Analyst*, 1942, 132).

The use of pH determinations as a means of controlling the purity of substances of pharmaceutical use is exemplified by the following instances taken from the B.P. 1932. *Ether*.—The aqueous extract has a pH of 4.9 to 5.2. *Potassium antimonyltartrate* and *sodium antimonyltartrate* are controlled for acidity and alkalinity by the amount of alkali or acid necessary to adjust the pH of a solution to 4.5. *Solution of ammonium acetate* is required to have a pH of 7.0 to 8.0. *Sodium and potassium bicarbonates* in 1% w/v aqueous solution are required to have a pH of 8.6. The assay of *sodium phosphate* involves titration to a definite pH value and the amount of acid necessary to adjust the pH of a solution of *sodium acid phosphate* to this same value limits the amount of disodium phosphate present.

Approximate pH of Some Common Substances

The following table gives the approximate pH of aqueous solutions, of the strength indicated, of some substances commonly met with in pharmacy.

| SUBSTANCE | MOLAR STRENGTH OF SOLUTION | w/v CONCENTRATION | APPROXIMATE pH |
|-------------------------|-------------------------------|--|----------------|
| Acid Acetic .. | 0.1 | 1 in 166 | 2.9 |
| Acid Ascorbic .. | 0.3 | 1 in 20 | 3.0 |
| Acid Benzoic .. | — | Saturated Solution | 2.8 |
| Acid Boric .. | 0.1 | 1 in 160 | 5.1 |
| Acid Citric .. | 0.1 | 1 in 48 | 2.1 |
| Acid Hydrochloric .. | 0.1 | 1 part HCl in 275 | 1.0 |
| Acid Mandelic .. | 0.66 | 1 in 10 | 1.8 |
| Acid Nicotinic .. | 0.08 | 1 in 100 | 3.5 |
| Acid Nitric .. | 0.1 | 1 part HNO ₃ in 158 | 1.1 |
| Acid Phosphoric .. | 0.1 | 1 part H ₃ PO ₄ in 100 | 1.5 |
| Acid Salicylic .. | — | Saturated Solution | 2.4 |
| Acid Sulphuric .. | 0.05 | 1 part H ₂ SO ₄ in 200 | 1.2 |
| Acid Tartaric .. | 0.1 | 1 in 66 | 1.9 |
| Acid Trichloroacetic .. | 0.1 | 1 in 60 | 1.2 |
| Acridine .. | — | 1 in 1000 | 2.5 |
| Alum (Ammonia) .. | 0.05 | 1 in 44 | 4.6 |
| Alum (Potash) .. | 0.1 | 1 in 21 | 4.2 |
| Ammonia .. | 0.1 | 1 part NH ₃ in 580 | 11.3 |
| Ammonium Bromide .. | 0.1 | 1 in 100 | 4.6 |
| Ammonium Chloride .. | 0.1 | 1 in 188 | 4.6 |
| Amylocaine .. | — | — | — |
| Hydrochloride .. | 0.19 | 1 in 20 | 5.0 |
| Aneurine .. | — | — | — |
| Hydrochloride .. | 0.14 | 1 in 20 | 3.5 |
| Apomorphine .. | — | — | — |
| Hydrochloride .. | 0.01 | 1 in 300 | 4.8 |
| Arsenic Trioxide .. | 0.05 | 1 in 100 | 5.5 |
| Atropine .. | — | Saturated Solution | 9.5 |
| Atropine Sulphate .. | 0.014 | 1 in 100 | 5.4 |
| Borax .. | 0.1 | 1 in 26 | 9.2 |
| Caffeine and .. | — | — | — |
| Sodium Benzoate .. | — | 1 in 25 | 7.4 |
| Calcium Chloride .. | 0.2 | 1 in 22 | 6.5 to 7.5 |
| (crystalline) .. | — | — | — |
| Calcium Gluconate .. | 0.22 | 1 in 10 | 6.5 to 7.5 |
| Calcium Hydroxide .. | — | Saturated Solution | 13.5 |
| Calcium Lactate .. | 0.13 | 1 in 25 | 6.0 to 7.0 |
| Carbachol .. | 0.003 | 1 in 2000 | 7.5 |
| Cocaine .. | — | — | — |
| Hydrochloride .. | 0.1 | 1 in 30 | 4.5 |
| Codeine .. | — | Saturated Solution | 9.8 |
| Codeine Phosphate .. | 0.1 | 1 in 24 | 4.5 |
| Emetine .. | — | — | — |
| Hydrochloride .. | 0.03 | 1 in 50 | 5.6 |
| Ephedrine .. | 0.03 | 1 in 200 | 10.8 |
| Ephedrine .. | — | — | — |
| Hydrochloride .. | 0.025 | 1 in 200 | 5.1 |
| Hexobarbitone .. | — | — | — |
| Soluble .. | 0.38 | 1 in 10 | 10.5 |
| Histamine Acid .. | — | — | — |
| Phosphate .. | 0.0033 | 1 in 1000 | 5.0 |
| Homatropine .. | — | — | — |
| Hydrobromide .. | 0.03 | 1 in 100 | 4.4 |
| Iodoxy .. | 1.5 | 1 in 1.3 | 9.0 |

| SUBSTANCE | MOLAR STRENGTH OF SOLUTION | w/v CONCENTRATION | APPROXI- MATE pH |
|------------------------|-------------------------------|--------------------|---------------------|
| Leptazol | 0.7 | 1 in 10 | 7.0 |
| Magnesium Sulphate | 0.2 | 1 in 20 | 6.0 to 7.0 |
| Mepacrine | | | |
| Methanesulphonate | 0.16 | 1 in 10 | 6.5 to 7.5 |
| Mersalyl | 0.2 | 1 in 10 | 8.5 |
| Methyl | | | |
| Hydroxybenzoate .. | 0.006 | 1 in 1000 | 6.5 |
| Morphine | | | |
| Hydrochloride .. | 0.028 | 1 in 100 | 5.6 |
| Morphine Sulphate.. | 0.066 | 1 in 20 | 6.0 |
| Nikethamide | 1.4 | 1 in 4 | 7.5 |
| Phenol | 0.26 | 1 in 40 | 5.0 |
| Phenylmercuric | | | |
| Nitrate | 0.0016 | 1 in 1000 | 4.0 |
| Phenytol Soluble .. | 0.0035 | 1 in 1000 | 10.0 |
| Physostigmine | | | |
| Salicylate | 0.015 | 1 in 100 | 6.5 |
| Pilocarpine Nitrate .. | 0.037 | 1 in 100 | 4.8 |
| Potassium Acetate .. | 0.1 | 1 in 100 | 9.7 |
| Potassium Acid | | | |
| Tartrate | 0.021 | 1 in 250 | 5.5 |
| Potassium | | | |
| Antimonytartrate .. | 0.06 | 1 in 50 | 4.2 to 4.8 |
| Potassium | | | |
| Bicarbonate | 0.1 | 1 in 100 | 8.2 |
| Potassium Bromide.. | 0.2 | 1 in 42 | 6.5 to 8.0 |
| Potassium Carbonate | 0.1 | 1 in 72 | 11.6 |
| Potassium Chlorate.. | 0.205 | 1 in 40 | 7.0 |
| Potassium Citrate .. | 0.2 | 1 in 16 | 8.0 to 8.5 |
| Potassium | | | |
| Hydroxide | 0.1 | 1 in 180 | 13.5 |
| Potassium Iodide .. | 0.2 | 1 in 30 | 7.0 to 9.0 |
| Potassium Nitrate .. | 0.2 | 1 in 50 | 6.5 to 7.5 |
| Procaine | | | |
| Hydrochloride .. | 0.1 | 1 in 36 | 6.0 |
| Proflavine Sulphate.. | 0.007 | 1 in 500 | 4.5 |
| Quinidine Sulphate .. | 0.0062 | 1 in 200 | 6.4 |
| Quinine | — | Saturated Solution | 8.8 |
| Quinine Bisulphate.. | 0.0725 | 1 in 25 | 3.5 |
| Quinine | | | |
| Dihydrochloride .. | 0.1 | 1 in 25 | 2.6 |
| Quinine | | | |
| Hydrobromide .. | 0.09 | 1 in 25 | 6.4 |
| Quinine | | | |
| Hydrochloride .. | 0.1 | 1 in 25 | 6.4 |
| Quinine Sulphate .. | — | Saturated Solution | 6.2 |
| Quinine and Urea | | | |
| Hydrochloride .. | — | 1 in 20 | 3.1 |
| Silver Nitrate | 0.03 | 1 in 200 | 6.5 to 7.5 |
| Sodium Acetate | 0.1 | 1 in 74 | 9.7 |
| Sodium Acid | | | |
| Phosphate | 0.1 | 1 in 64 | 4.5 |
| Sodium | | | |
| Antimonytartrate .. | 0.06 | 1 in 50 | 4.2 to 4.8 |
| Sodium Benzoate .. | 0.1 | 1 in 70 | 8.0 |
| Sodium Bicarbonate | 0.1 | 1 in 20 | 8.2 |
| Sodium Bromide | 0.2 | 1 in 48 | 6.5 to 8.0 |
| Sodium Carbonate .. | 0.1 | 1 in 35 | 9.6 |
| (crystalline) | | | |
| Sodium Chloride .. | 0.2 | 1 in 86 | 6.7 to 7.3 |
| Sodium Citrate | 0.2 | 1 in 17 | 8.0 to 8.5 |

| SUBSTANCE | MOLAR STRENGTH OF SOLUTION | w/v CONCENTRATION | APPROXI- MATE pH |
|--------------------------------|-------------------------------|-------------------|---------------------|
| Sodium Hydroxide .. | 0.1 | 1 in 250 | 13.5 |
| Sodium Iodide .. | 0.2 | 1 in 33 | 8.0 to 9.5 |
| Sodium Metabisulphite .. | 0.052 | 1 in 100 | 5.0 |
| Sodium Phosphate .. | 0.1 | 1 in 28 | 9.5 |
| Sodium Potassium Tartrate .. | 0.2 | 1 in 18 | 7.0 to 8.0 |
| Sodium Salicylate .. | 0.2 | 1 in 30 | 5.0 to 6.0 |
| Sodium Sulphate .. | 0.2 | 1 in 16 | 6.0 to 7.5 |
| Sodium Thiosulphate .. | 0.2 | 1 in 20 | 6.5 to 8.0 |
| Soluble Barbitone .. | 0.1 | 1 in 48 | 9.4 |
| Stibophen .. | 0.07 | 1 in 16 | 6.5 |
| Strychnine Hydrochloride .. | 0.025 | 1 in 100 | 5.4 |
| Sulphacetamide Soluble .. | 1.5 | 1 in 3 | 7.5 |
| Sulphanilamide .. | 0.012 | 1 in 500 | 7.0 |
| Sulphapyridine Soluble .. | 0.17 | 1 in 20 | 10.0 to 11.0 |
| Sulphathiazole Soluble .. | 0.18 | 1 in 20 | 9.0 to 10.0 |
| Suramin .. | 0.007 | 1 in 100 | 6.2 to 6.8 |
| Theophylline .. | 0.25 | 1 in 20 | 6.5 |
| Theophylline Sodium Acetate .. | — | 1 in 50 | 9.0 |
| Tribromoethyl Alcohol .. | 0.035 | 1 in 100 | 5.5 |
| Tryparsamide .. | 0.66 | 1 in 5 | 7.5 |

OXIDATION-REDUCTION POTENTIALS

The term oxidation is used not only to indicate addition of oxygen or removal of hydrogen but also to indicate removal of electrons. This latter process is common to all oxidations in solution; thus, the oxidation of ferrous to ferric iron may be represented:—

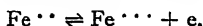


Oxidised form + electrons \rightleftharpoons reduced form.

An oxidising agent, therefore, reacts by taking up electrons, becoming itself reduced. If, therefore, an unattackable electrode such as platinum is dipped into a solution containing an oxidising agent, the latter will tend to take up electrons from the metal thereby causing the metal to become positively charged with respect to the solution. Similarly, on being placed in a solution of a reducing agent the metal will tend to take up electrons, thereby becoming negatively charged with respect to the solution. The oxidising power of a solution is measured not merely by its concentration in terms of the oxidising substance present but also in terms of the positive potential acquired by an unattackable

electrode placed in the solution. The more positive the potential acquired the greater is the oxidising power of the solution, and the more negative the potential the greater the reducing power.

In order to obtain a definite potential from the solution both oxidised and reduced forms must be present, and each must be convertible into the other as a result of an infinitesimally small change of the equilibrium potential in one direction or the other. In the oxidation of ferrous to ferric iron, provided a mere trace of ferric iron is present, an equilibrium exists between the reduced and oxidised forms and electrons, and a platinum electrode, by taking up the electrons, would acquire a negative potential with respect to the solution. The value of that potential indicates the relative amounts of reduced and oxidised states present:—



In general, where n electrons are required to convert one molecule of oxidised form to one molecule of reduced form in the equilibrium

Oxidised form + n electrons \rightleftharpoons Reduced form,
the potential acquired by an unattackable electrode is given by

$$E = E_0 + \frac{0.0002T}{n} \log_{10} \frac{[\text{oxidised form}]}{[\text{reduced form}]},$$

where E_0 is a constant for the particular system and is known as the normal or standard oxidation-reduction potential of the system, and T is the absolute temperature. When the concentrations of oxidised and reduced forms are equal the observed potential will be equal to the normal oxidation-reduction potential of the system.

By varying the relative amounts of oxidised and reduced forms present the actual potential may be made very large or very small. If the normal potential of the system is known it is possible to determine the relative proportions of oxidised and reduced forms present from the observed value of the potential.

In the reduction of permanganate to manganous salt in acid solution the equilibrium existing is expressed by:—



The potential of the system is given by

$$E = E_0 + \frac{0.0002T}{5} \log_{10} \frac{[\text{MnO}_4'] [\text{H}^+]^8}{[\text{Mn}^{++}]}.$$

The potential acquired by this system is dependent on the eighth power of the hydrogen ion concentration.

The following table gives the standard potentials of some of the more common systems.

Standard Oxidation-Reduction Potentials at 25° (Volts)

| | | | |
|---|-------|---|-------|
| $\text{Ce}^{++++}, \text{Ce}^{+++}$ | 1.55 | $\text{Fe}^{++}, \text{Fe}^{+}$ | 0.748 |
| $\text{MnO}_4' + 8\text{H}^+, \text{Mn}^{++}$ | 1.48 | $\text{Fe}(\text{CN})_6^{---}, \text{Fe}(\text{CN})_6^{----}$ | 0.486 |
| $\text{IO}_3' + 6\text{H}^+, \frac{1}{2}\text{I}_2$ | 1.197 | $\text{Ti}^{+++}, \text{Ti}^{++}$ | 0.06 |
| $\text{Hg}^{++}, \text{Hg}_2^{++}$ | 0.901 | $\text{Sn}^{+++}, \text{Sn}^{++}$ | -0.15 |

In general, any system higher in the table will oxidise one lower down and, similarly, any system will reduce one above it; the actual extent of oxidation or reduction can be determined quantitatively by means of the standard potentials. Consider, for example, the reaction between the systems ferric-ferrous and stannic-stannous. The extent to which the process



will occur can be calculated from the requirement that reaction between any two systems will continue until their respective potentials are equal. Repre-

senting the final, or equilibrium concentrations by the subscript e and using the standard potentials given in the table it follows that at equilibrium,

$$E_0 + 0.0002T \log_{10} \frac{[\text{Fe}^{\cdots\cdots}]_e}{[\text{Fe}^{\cdots}]_e} = E'_0 + \frac{0.0002T}{2} \log_{10} \frac{[\text{Sn}^{\cdots\cdots}]_e}{[\text{Sn}^{\cdots}]_e}.$$

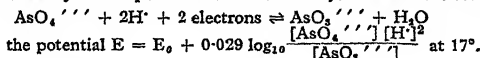
$$\text{Hence } 0.748 + 0.0002T \log_{10} \frac{[\text{Fe}^{\cdots\cdots}]_e}{[\text{Fe}^{\cdots}]_e} = -0.15 + 0.0001T \log_{10} \frac{[\text{Sn}^{\cdots\cdots}]_e}{[\text{Sn}^{\cdots}]_e}.$$

From which it can be calculated that at 25°

$$[\text{Fe}^{\cdots\cdots}]_e^2 [\text{Sn}^{\cdots\cdots}]_e = 10^{32} \times [\text{Fe}^{\cdots}]_e^2 [\text{Sn}^{\cdots}]_e.$$

The factor 10^{32} indicates that the product of the concentrations of ferric and stannous ions is negligibly small compared with that of ferrous and stannic ions. Hence for all practical purposes stannous ions completely reduce ferric ions to ferrous, a process often used in the determination of ferric salts by reduction with stannous chloride and titration of the ferrous ions with dichromate.

As an example of the influence of hydrogen ion concentration on the potential of a system of analytical importance consider the system arsenate-arsenite:—

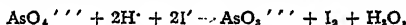


The value of E_0 is 0.57 volt in strongly acid solution and if the ratio of arsenate to arsenite is unity, this will be the observed potential. In a solution of pH 9, however, the corresponding potential is 0.15 volt.

The system



has a normal potential of 0.4 volt. In strongly acid solution, therefore, the arsenate-arsenite system will oxidise iodine-iodide and the reaction occurring will be



a process which is complete within the limits of analytical accuracy. Hence arsenate can be determined by adding iodide to a strongly acid solution and titrating the liberated iodine.

It will be seen from the above figures that, in a solution of pH 9, the iodine-iodide system has the higher potential and it will therefore oxidise arsenite to arsenate.



The reaction will be quantitative provided the solution is maintained at pH 9 a condition effected by adding sodium bicarbonate which removes the acid as formed.

Potentiometric Titrations.

Titration involving the use of oxidising agents can be followed by potentiometric methods (*vide supra*, Hydrogen Ion Concentration). Providing the oxidising agent used has a normal potential at least 0.3 volt more positive than that of the system to be oxidised, then by measuring the potential after each addition of portions of oxidising agent and plotting the observed potential against volume of titrant added the end-point will be indicated by a sharp vertical rise in the curve. This indicates the comparatively large increase in potential that occurs when the oxidising agent is present in very slight excess.

An alternative simple process depends on the fact that although a platinum electrode indicates the potential of the oxidation-reduction system, other metals such as tungsten and palladium

do not do so. At the end-point of the titration, therefore, the platinum electrode potential changes suddenly but that of a tungsten electrode in the same solution remains almost unchanged. The platinum and tungsten electrodes are placed in the solution to be titrated and connected with a galvanometer through a tapping key. The titrant is added gradually and the galvanometer deflection noted on closing the circuit; the end-point is indicated by a large deflection, since the E.M.F. of the cell is then changing rapidly.

Determination of Oxidation-Reduction Potential by means of Indicators.

If the oxidised and reduced forms of a substance have different colours, e.g., methylene blue, it may be used, within limits, as an indicator of oxidation-reduction potentials, much as an ordinary indicator is used for the determination of hydrogen ion concentration (*vide supra*). Mixtures of the two forms in different proportions will have different depths of colour—corresponding to definite ratios of oxidised to reduced forms. The depth of colour, therefore, indicates the oxidation-reduction potential. Each indicator is applicable only over a definite range of potentials in the vicinity of its own normal potential; outside this range the colour will be entirely that of the oxidised or reduced form. When such an appropriate indicator is placed into an oxidation-reduction system, it will enter into an equilibrium in which its oxidation-reduction potential is the same as that of the system; from the colour of the solution the value of the potential may be determined. The indicators used must be standardised by actual electrode potential measurements and the hydrogen ion concentration controlled, or the colour may be compared with that given by the same indicator to “poised” solutions which are resistant to changes of oxidation-reduction potential. These “poised” solutions are comparable with “buffer” solutions used in measurement of hydrogen ion concentration. (For formulæ of poised solutions see Hirsch and Ruter, *Z. anal. Chem.*, 1926, 69, 193.)

rH Value.—The rH value is a concise method of expressing the oxidising or reducing tendency of a system and is of value, for example, in biological work where the pH of the systems dealt with does not differ markedly from pH 7.

For any system in which the reduced form differs from the oxidised form by two atoms of hydrogen only, as in the equilibrium:—

Oxidised form + 2H^+ + 2 electrons \rightleftharpoons Reduced form, the potential is given by:

$$E = E_0 + 0.0001T \log_{10} \frac{[\text{oxidised form}]}{[\text{reduced form}]} + 0.0002T \log_{10} [\text{H}^+].$$

The potential of an ordinary hydrogen electrode, with the gas pressure p atmospheres, is given by

$$E_{\text{H}} = 0.0001T \log_{10} \frac{1}{p} + 0.0002T \log_{10} [\text{H}^+].$$

Consider the oxidation-reduction electrode in equilibrium with the theoretical hydrogen electrode, with gas at a pressure p determined by the values of E_0 and

of the ratio $\frac{[\text{oxidised form}]}{[\text{reduced form}]}$, expressing this hypothetical pressure p in terms of a quantity rH such that

$$rH = \log_{10} \frac{1}{p}$$

$$\begin{aligned} \text{Then } E = E_H &= (0.0001T) rH + (0.0002T) \log_{10} [H^+] \\ &= (0.0001T) rH - (0.0002T) pH. \end{aligned}$$

At 17° this becomes

$$\begin{aligned} E &= 0.029 (rH - 2 pH) \\ \text{or } rH &= \frac{E}{0.029} + 2 pH. \end{aligned}$$

rH is therefore defined as the logarithm of the reciprocal of the pressure of hydrogen gas, expressed in atmospheres, in a hydrogen electrode with which the oxidation-reduction system is theoretically in equilibrium.

Since the hydrogen pressures required are usually small, rH may range usually from 0 up to about 30.

The conception rH is employed in expressing oxidation-reduction potentials in biological work and also as an indication of the range of potential for which oxidation-reduction indicators may be employed.

It will be seen from the expression above that the rH of a well-defined oxidation reduction system can and does change with the pH of the system.

Applications.

Among applications of oxidation-reduction potentials are: the study of the accuracy of titration methods; the investigation of biologically important oxidation-reduction systems such as glutathione which can be shown to be a powerfully reducing system playing an important part in the oxidation of fats, the reduced (cystine) form of glutathione being oxidised in the presence of oxygen, and the resulting cysteine derivative reacting with the oxidisable fats; the study of enzymes and of the effects of bacteria; the methylene blue test for milk (*vide* p. 792), which depends on the increase in the reducing power of milk due to increase in the bacterial content.

Other references: Oxidation-Reduction Potentials and their Applications, by S. Glasstone, 1937, Institute of Chemistry, London; *The Oxidation States of the Elements and their Potentials in Aqueous Solutions*, by W. M. Latimer, 1938, New York; *Electrolytic Oxidation and Reduction*, by Glasstone and Hickley, 1935, London; *Electrochemistry of Solutions*, by S. Glasstone, 2nd Edn., 1937, London; *Oxidation-Reduction Potentials*, by Michaelis, 1930, London.

POLAROGRAPHIC ANALYSIS

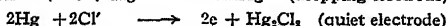
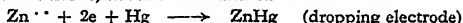
When an electrolyte solution is electrolysed in a cell consisting of a dropping mercury electrode and a second non-polarisable electrode it is possible to determine from the resulting current-voltage curve both the nature and concentration of the reducible or oxidisable substance or substances present. This is the essential feature of polarographic analysis invented by Jaroslav Heyrovsky. Heyrovsky and Shikata (*Rec. trav. chim.*, 1925, **44**, 496) described an instrument called the polarograph by means of which dropping electrode current-voltage curves could be obtained automatically and recorded photographically, the recorded curves being termed polarograms. In favourable cases it is possible to detect and determine simultaneously as many as five or six different substances from a single polarogram. The method is specially

suitable for small concentrations (10^{-6} to 10^{-2} molar) and since the analysis can be performed with a very small volume of solution, mere traces of substances can be determined. Since also quantitative and qualitative analyses are obtained simultaneously in a single operation, the polarographic method is becoming a valuable new addition to present analytical methods.

The Current-Voltage Curve. Electrolysis may be defined as the occurrence of chemical reactions, under the influence of an electromotive force at electrodes immersed in solutions. Electrolytic reactions are characterised by the flow of an electric current between the solution and the electrodes, and the magnitude of the current is a measure of the net rate of the electrode reactions. In any electrolysis cell the reaction at one electrode is an electroreduction (at the cathode), and an exactly equivalent amount of electro-oxidation occurs at the other electrode (anode). The curve obtained by plotting the electromotive force applied to a cell against the resulting current is known as a current-voltage curve. A typical arrangement for obtaining current-voltage curves with the dropping mercury electrode consists of an electrolysis cell containing the solution to be analysed above a pool of mercury at the bottom of the cell which serves as the second electrode.

The Dropping Electrode. This consists of a drawn out capillary tube, whose internal diameter at the tip is about 0.03 millimetre, connected to a reservoir of mercury. Mercury drops issue from the capillary, fitted below the surface of the solution in the cell, at the rate of one drop every two to four seconds. The drops are very small, having a maximum diameter at the breaking point of about 0.5 millimetre. The mercury in the dropping electrode is connected through a galvanometer to a resistance across which a battery is connected, the dropping mercury electrode and the negative pole of the battery being attached to the same point on the resistance. The anode of the cell (the pool of mercury below the solution being examined) is connected to the resistance in such a manner that the electromotive force applied to the electrolysis cell can be varied from zero to the maximum of the battery.

Technique. The current-voltage curves are obtained by gradually increasing the applied electromotive force and noting the current indicated by the galvanometer. The current is ordinarily quite small, seldom exceeding 50 microamperes (5×10^{-5} amperes). Since oxygen is readily reduced at the dropping electrode, and usually interferes with the current-voltage curves of other substances it is generally necessary to remove dissolved air from the solution to be electrolysed by bubbling an inert gas (nitrogen, hydrogen or carbon dioxide) through the cell before, but not during, the electrolysis. When, for example, an air-free solution of 0.0013 M zinc sulphate in 0.1 N potassium chloride is electrolysed in such a cell, the current flowing is exceedingly small until the decomposition potential is reached at an applied electromotive force of about 1.0 volts. As the applied electromotive force is increased, continuous electrolysis occurs consisting of a discharge of zinc ions at the dropping mercury cathode, to form an extremely dilute zinc amalgam, and solution of mercury at the anode, which forms calomel.



The current flowing increases sharply as electrolysis begins, but it does not increase indefinitely with increasing applied electromotive force, and gradually approaches a limiting value, finally becoming constant and independent of the increase in the applied electromotive force. Under optimum conditions and with all other factors constant, the limiting current is directly proportional to the concentration of the electroreducible substance. This fact is the basis of quantitative polarographic analysis. The limiting current is caused by a virtually complete state of concentration at the dropping electrode. As a result of the discharge process, the concentration of the reducible material is diminished close to the surface of the dropping electrode and this loss is compensated by diffusion of a fresh supply of reducible material from the bulk of the solution. The rate of diffusion depends directly on the difference in concentration between that diminished in the surface layer and that in the bulk of the solution. As the electromotive force is increased above the decomposition potential and

the current increases, the average concentration of the reducible substance at the surface of the mercury drops decreases, and the rate of diffusion is correspondingly increased. As the applied electromotive force is further increased the concentration at the surface of the mercury drops becomes so small compared with the concentration in the bulk of the solution that the difference in concentration approaches a constant average value, equal simply to the concentration in the solution and hence the rate of diffusion also becomes constant. From this point on, the amount of material discharging, and hence the current, becomes constant and practically independent of further increase in the applied electromotive force. With the dropping mercury electrode, the current at each value of the applied electromotive force is not constant but oscillates between a minimum and a maximum value due to the periodic change in area as each mercury drop grows and falls. With a sensitive galvanometer of relatively long period the observed oscillations are not very large and the average current can be measured readily with a precision of better than 1%.

The Half-Wave Potential. The decomposition potential of a given solution is characteristic of the particular electroreducible substance present. Even more characteristic is the so-called "half-wave potential" which, as its name implies, is the value of the potential of the dropping electrode against an external reference electrode at that point on a current-voltage curve where the current is equal to half its limiting value. In contrast to the decomposition potential which depends on the concentration of the reducible substance, the half-wave potential is in general independent of the concentration of the reducible substance provided that the composition of the solution with respect to foreign salts is kept constant. Qualitative polarographic analysis is based on the characterisation of the half-wave potentials on the current-voltage curves of solutions. The half-wave potentials of many simple metal ions are shifted to more negative values when complex-forming salts are added to the solution. This fact is often used to separate the polarographic waves of two metal ions which would otherwise coincide.

As a guide to the variations in the half-wave potential (E_1) of some of the common elements as their ions the following table compiled from data collected by Kolthoff and Lingane (*Polarography*, New York, 1941) is of interest, values of E_1 being given by reference to the normal hydrogen electrode at 25°.

| SUBSTANCE | SUPPORTING ELECTROLYTE | E_1 IN VOLTS ON NORMAL HYDROGEN SCALE |
|-------------|--|--|
| Aluminum .. | 0.05 N/1 BaCl ₂ or KCl .. | -1.504 |
| Antimony .. | Sb ⁺⁺⁺ in N/1 HCl or H ₂ SO ₄ .. | +0.006 |
| | Sb ⁺⁺⁺ in N/1 NaOH .. | -0.924 |
| | Antimonyl tartrate ion in 0.1 M K ₂ SO ₄ + 0.002 N NaOH without excess tartrate .. | -0.694 |
| Barium .. | 0.1 N LiCl or MgCl ₂ .. | -1.654 |
| Bismuth .. | Bi ⁺⁺⁺ in N/1 HCl .. | +0.196 |
| | Bi ⁺⁺⁺ in N/1 HNO ₃ .. | +0.146 |
| Chlorine .. | 0.001 N Cl ⁻ in 0.1 N KNO ₃ .. | +0.496 |
| Chromium .. | CrO ₄ ²⁻ in N/1 NaOH .. | -0.604 |
| Copper .. | N/1 NH ₄ OH + N/1 NH ₄ Cl .. | +0.046 (Cu ⁺⁺ to Cu ⁺) |
| | | -0.234 (Cu ⁺ to Cu) |
| | Fe ⁺⁺ in 0.1N KCl or BaCl ₂ .. | -1.054 |
| Iron .. | Fe ⁺⁺⁺ and/or Fe ⁺⁺ in M/1 K ₂ CO ₃ .. | +0.006 (Fe ⁺⁺⁺ ⇌ Fe ⁺⁺) |
| | Fe ⁺⁺⁺ and/or Fe ⁺⁺ in alkaline tartrate solution .. | -0.654 |
| Lead .. | N/1 KNO ₃ or HNO ₃ .. | -0.159 |
| | N/1 KCN .. | -0.474 |
| Nitrogen .. | 0.001 N CNS ⁻ in 0.01 N KNO ₃ .. | +0.396 |
| | 0.001 N CN ⁻ in 0.01 N NaOH .. | -0.154 |
| Oxygen .. | OH ⁻ in 0.1 N to N/1 KNO ₃ .. | +0.276 |
| Silver .. | KAg(CN) ₂ without excess cyanide or other supporting electrode .. | -0.054 |
| | N/1 KNO ₃ .. | -0.766 |
| Zinc .. | 2N NH ₄ OH + 2N NH ₄ Cl .. | -1.184 |

Applications. Since so many diverse types of substances are subject to electrolytic reduction and oxidation, the scope of the polarographic method is potentially very great. In inorganic analysis it has been applied to the determination of practically all of the common metals and other reducible ions. Typical examples of procedures that have already been developed include the rapid determination of copper, zinc, iron, lead and nickel in brass and similar alloys, the determination of copper, nickel, cobalt, manganese and chromium in steel, and the determination of aluminium, zinc, manganese and lead in commercially important manganese alloys. The method is particularly suited to the determination of traces of impurities in commercial chemicals and many applications of this possibility have been made, e.g., the rapid determination of traces of lead in citric acid, the determination of small amounts of bromate and iodate in chlorates and the determination of small amounts of copper and nickel in manganese salts.

Oxygen is readily reduced at the dropping electrode and gives a well-defined wave. This fact has been applied to the determination of oxygen in gases and for the determination of oxygen in biochemical studies of the respiration rates of various organisms.

A large variety of organic substances including various aldehydes and ketones, unsaturated acids, nitro and nitroso compounds, azo and diazo compounds, and quinones are reducible at the dropping electrode and yield well-defined waves. With further exploration of its application to organic chemical problems polarographic analysis is likely to be of increasing value in biochemistry and biology as well as in general analysis. In some instances it is already possible to differentiate and determine the various members of a homologous series of compounds and in certain cases to differentiate between isomers.

Under ordinary conditions the accuracy of the polarographic method is about 2% for concentrations of 10^{-4} to 10^{-2} molar and about 5% for concentrations of 10^{-5} molar.

Amperometric Titration. The examination of the polarographic method has resulted in the development of a new method of electrometric titration, termed "amperometric titration," employing the dropping mercury electrode. This new technique is based on the measurement at a constant potential, of the change of the diffusion current of a reducible or oxidisable substance when it is titrated with a suitable reagent. The amperometric titration method is particularly useful for titrating relatively small concentrations of various metal ions and it is applicable in those cases where reversible indicator electrodes are not available for titration by classical potentiometric method.

Apparatus. Many types of complete apparatus are available commercially, based on the general principles already described. In some the gradual increasing of applied electromotive force is automatic and the current flowing through the solution being examined is recorded from the galvanometer on photosensitised paper moving in synchronised fashion with the stepping up of the electromotive force so that the resulting current-voltage curve is immediately obtained.

References

Some five hundred papers have been published on the analytical application of the dropping mercury electrode since 1925. In addition to those already mentioned the following are of particular interest:—

Traces of iodate may be determined in the presence of a large excess of nitrate, since the reduction potential of the latter in alkaline medium is more negative than that of iodate. The fact has been used to determine traces of iodate in Chile saltpetre. Traces of iodide have been determined polarographically by oxidising to iodate with chlorine water, removing the excess chlorine, and recording the iodate wave.—Rylich, *Coll. Czech. Chem. Comm.*, 1935, 7, 288.

The presence of and amounts of aldehyde forms in solutions of aldoses (glucose, galactose, mannose, xylose and ribose) has been shown by the polarographic method, the amount of aldehyde form increasing with increase in pH between 6 and 8.—Cantor and Peniston, *J. Amer. chem. Soc.*, 1940, 62, 2113

Ascorbic acid in fruit juices buffered at pH8 by phosphate has been determined polarographically.—Kodicek and Wenig, *Nature, Lond.*, 1938, 38; see also M. M. Kirk, *Industr. Engng Chem. (anal. Edn.)*, 1941, 13, 625.

Testosterone, progesterone, and desoxycorticosterone have been determined by the polarographic method, though the method determines the keto group associated with the conjugated double bond $O = C - C = C -$ rather than the specific substance.—Eisenbrand and Picher, *Z. physiol. chem.*, 1939, 260, 83.

Saccharin is quantitatively determined by the polarographic method, yielding well-defined waves in acid, neutral or alkaline media. A solution in 0.05N hydrochloric acid and 0.05N potassium chloride is recommended.—Pech, *Coll. Czech. Chem. Comm.*, 1934, 6, 126.

Polarographic examination has been fairly widely examined in serological cancer diagnosis and though still more work is necessary the results so far obtained cannot be ignored (see Brdicka, *Nature, Lond.*, 1937, 330, 1020; C. R. soc. Biol., Paris, 1938, 128, 54; Bergh, Henriques and Schonsbon, *Nature, Lond.*, 1938, 751; Meyer-Heck, *Z. Krebsforsch.*, 1939, 49 142; Tropp, *Klin. Wschr.*, 1938, 17, 1141; Walker and Reimann, *Amer. J. Cancer*, 1939, 37, 585; Koltjar and Podrouzek, *Nature, Lond.*, 1938, 872; Dodds, *Lancet*, ii/1938, 351).

Riboflavin may be determined polarographically in the presence of aneurine, nicotinic acid, pyridoxin, and pantothenic acid at pH7 especially if calibration data are obtained for the capillary used.—Lingane and Davis, *J. biol. Chem.*, 1941, 137, 567.

Many applications have been made of the polarographic method to determination of metals in biological materials.—Kolthoff and Lingane, *Polarography* (New York, 1941), p. 423.

Lead may be quantitatively determined by dichromate amperometrically even in very dilute solutions.—Kolthoff and Pan, *J. Amer. chem. Soc.*, 1939, 61, 3402.

Aqueous solutions of sulphates at concentrations of 0.01M or greater can be titrated amperometrically with 0.1M lead nitrate with an accuracy of ± 0.3 per cent.—Kolthoff and Pan, *J. Amer. chem. Soc.*, 1940, 62, 3332.

Vitamin K and related compounds may be determined polarographically.—Hershberg, Wolfe and Filser, *J. Amer. chem. Soc.*, 1940, 62, 3516.

The polarographic method has been applied to the analysis of aluminium and its alloys.—A. C. Coates and R. Smart, *J. Soc. chem. Ind.*, 1941, 60, 249.

Organic mercapto- and mercaptoamino- acids and cystine- and cystine-containing proteins give characteristic current-voltage curves in cobalt buffer solutions. This method has been applied to the determination of such compounds in urine.—G. Reed, *J. biol. Chem.*, 1942, 142, 61.

Phosphorus in biological material (food, faeces and urine) may be determined by an indirect polarographic method, molybdate concentration being determined polarographically before and after precipitation of the phosphate ions as ammonium phosphomolybdate.—A. Stern, *Industr. Engng Chem. (anal. Edn.)*, 1942, 14, 74.

Other references: H. Hohn, *Chemische Analysen mit dem Polarograph*, Berlin, 1937; J. Maas, *De Polarografische Methode met de druppelende Kuiklectrode ten Dienste van het Pharmaceutisch Onderzoek*, Amsterdam, 1937. See also *Ann. Rep. Chem. Soc., Lond.*, 1938, 389-394; Kolthoff and Lingane, *Chem. Rev.*, 1939, 24, 1-94; A. C. Coates and R. Smart, *Chem. & Ind.*, 1941, 778; O. H. Müller, *J. Chem. Educ.*, 1941, 18, 65-72; 111-115; 172-177; 227-234; 320-329.

For description of available apparatus see R. H. Müller, *Industr. Engng Chem. (anal. Edn.)*, 1941, 13, 667.

INDICATORS

The following indicators are those most generally used for volumetric analysis and for the colorimetric determination of the hydrogen ion concentration of solutions.

| INDICATOR | COLOUR CHANGE | STRENGTH AND SOLVENT | REMARKS |
|--|---|---|---|
| Alkali Blue <i>Syn.</i> Nicholson's Blue. A mixture of the sodium sulphonates of phenylated rosaniline and para-rosaniline | From blue to red with strong alkali in alcoholic solution | 0.1% in alcohol (90%) | Used in the alkali limit test of the <i>B.P.</i> for Liquor Cresolis Saponatus. |
| Bromocresol Green (Tetra-bromo- <i>m</i> -cresol-sulphone-phthalein) | *pH 3.6 yellow pH 5.2 blue | 0.1 g. warmed with 2.9 ml. of N/20 NaOH and 5 ml. of alcohol (90%) until dissolved and diluted with alcohol (20%) to 250 ml. | Used for titrating phosphoric acid and disodium phosphate. Gives a green colour at pH 4.5 which corresponds to the formation of sodium acid phosphate. |
| Bromocresol Purple (Dibromo- <i>o</i> -cresol-sulphone-phthalein) | pH 5.2 yellow pH 6.8 purple | 0.1 g. warmed with 3.7 ml. of N/20 NaOH and 5 ml. of alcohol (90%) until dissolved, and diluted with alcohol (20%) to 250 ml. | "This is the most trustworthy indicator for quinine."— <i>J. Amer. chem. Soc.</i> , 1922, 2156. Used for determination of the pH of solutions. |
| Bromophenol Blue (Tetra-bromo-phenol-sulphone-phthalein) | pH 2.8 yellow pH 4.6 purple | 0.1 g. warmed with 3 ml. of N/20 NaOH and 5 ml. of alcohol (90%) until dissolved and diluted with alcohol (20%) to 250 ml. | Methyl red, methyl orange or cochineal give low results for morphine, while bromophenol blue gives a satisfactory value and end-point. This is also true for atropine and mydriatic residues.— <i>J. chem. Soc. Abstr.</i> , ii/1922, 885; also <i>Pharm. J.</i> , i/1921, 470. Used for pH determinations. |

*pH 3.6 means a hydrogen ion concentration of $10^{-3.6}$ g. per litre (see p. 581), when bromocresol green is yellow. The addition of alkali to lower the hydrogen concentration to 10^{-7} changes the colour. Thus with acids this indicator is yellow and with alkalis purple.

| INDICATOR | COLOUR CHANGE | STRENGTH AND SOLVENT | REMARKS |
|---|---|--|---|
| Bromo-thymol Blue (Dibromo-thymol-sulphone-phthalein) | pH 6 yellow pH 7.6 blue | 0.1 g. warmed with 3.2 ml. of N/20 NaOH and 5 ml. of alcohol (90%) until dissolved, and diluted with alcohol (20%) to 250 ml. | Used for determination of the pH of solutions. |
| Cochineal | pH 0—4 yellow pH 5 brown-pink pH 6 purple | B.P. employs the tincture (1 in 10) | Useless for organic acids. Sharp end-reaction with inorganic acids and bases by back titration. Suitable for solutions of the alkaline earths. Used for titrating alkaloids with mineral acids, but end-point not sharp. Cannot be used in presence of acetates or compounds of Fe or Al. |
| Congo Red (Sodium diphenyl-bisazobis-naphthyl-amine-4-sulphonate) | pH 3 blue pH 4 violet pH 5 scarlet | 0.5% in alcohol (25%) | Responds well to inorganic acids and inorganic bases. Responds to organic bases, but not good for titrating, e.g., quinine or atropine. |
| Cresol Red (<i>o</i> -cresol-sulphone-phthalein) | pH 7.2 yellow pH 8.8 red | 0.05 g. warmed with 2.65 ml. of N/20 NaOH and 5 ml. of alcohol (90%) until dissolved and diluted with alcohol (20%) to 250 ml. | Used for the determination of the pH of solutions. |
| Hæmatoxylin | Yellow with acids to green or purple with alkalis | 1% in alcohol (90%) | Responds to inorganic and organic acids. Responds to inorganic bases, and to organic, e.g., alkaloids. Occasionally used in alkaloidal titrations, e.g., quinine residues, yielding good end-points. |
| Dimethyl Yellow (Dimethyl-aminoazo-benzene) | pH 2.4 red pH 4 yellow | 0.2% in alcohol (90%) | |

| INDICATOR | COLOUR CHANGE | STRENGTH AND SOLVENT | REMARKS |
|--|--|---|---|
| Iodo-Eosin (Tetraiodo-fluorescein) | Red with alkalis in aqueous solution, yellow with acids in ether layer | 0.1% in alcohol (90%). 0.01% in water. Sometimes 0.01% in ether is used | Used for titrating minute quantities of alkali with N/100 or N/1000 acid and for small quantities of alkaloids which are alkaline to it. In use 10 ml. to 20 ml. of ether are added to the titration flask to form a layer above the liquid. Alkalis produce a red in the aqueous layer and acids a yellow in the ether layer. It is a poor indicator, e.g., with strychnine. Not suitable for ordinary titrations. |
| Lacmoid | pH 0—4 pink pH 5 violet pH 7 blue These changes are not sharp | 0.2% in alcohol (60%) | This indicator is somewhat less sensitive to CO_2 than litmus and is used similarly. |
| Litmus | pH 5 red pH 8 blue | Boil 10 g. for 1 hour with 40 ml. of alcohol (90%) and pour off the clear liquid; repeat the boiling, etc., twice with 30 ml. of alcohol (90%). Digest the washed litmus with 100 ml. of water and filter | CO_2 , if present must be removed by boiling. Suitable for inorganic acids and for lactic, oxalic and tartaric acids. Not suitable for weak acids and alkalis. Quinine, morphine and strychnine are neutral to it. The acid in their salts can be titrated, using litmus, as though no base were present.— <i>Pharm. J.</i> , i/1915, 135. The end-points are not good. Litmus solutions should be exposed to the air occasionally to preserve the colour. |
| Methyl Orange | pH 2.8 red pH 5 yellow | 0.04% in alcohol (20%) | Suitable for titrating strong mineral acids but cannot be used for organic acids. Alkaloids are alkaline to it, but end-point not good, e.g., in case of quinine. Alkali carbonates and bicarbonates can be titrated without boiling as this indicator is unaffected by CO_2 . Should not be used in alcoholic or boiling solutions. Acid phosphates, e.g., NaH_2PO_4 , are neutral to methyl orange. |

| INDICATOR | COLOUR CHANGE | STRENGTH AND SOLVENT | REMARKS |
|--|---|--|---|
| Methyl Red (<i>p</i> -Dimethyl-aminoazo-benzene- <i>o</i> -carboxylic acid) | pH 4.2 red pH 6.3 yellow | 0.025 g. warmed with 0.95 ml. of N/20 NaOH and 5 ml. of alcohol (90%) until dissolved and diluted with alcohol (25%) to 250 ml. | Is more sensitive than methyl orange and the colour change is sharper, but it is more sensitive to CO ₂ which must be removed by boiling. It is suitable for titrating ammonia or alkaloids but not for weak organic acids. Used for determination of the pH of solutions. |
| Naphthol-phthalein | pH 7.3 colourless pH 8.7 blue | 0.1% in alcohol | Used for determination of the pH of solutions. |
| Phenolphthalein | pH 8.3 colourless pH 10 red | 0.2 g. dissolved in 60 ml. of alcohol (90%) and diluted with water to 100 ml. | Usually employed for titrating inorganic and organic acids, and may be used in alcoholic or hot solutions. Some organic bases, e.g., the alkaloid atropine, are alkaline to this indicator but morphine, quinine and strychnine are not. Phenolphthalein must not be used in presence of ammonia or its salts, and is affected by CO ₂ . |
| Phenol Red | pH 6.8 yellow pH 8.4 red | 0.05 g. warmed with 2.85 ml. of N/20 NaOH and 5 ml. of alcohol (90%) until dissolved and diluted with alcohol (20%) to 250 ml. | Used for titrating weak organic acids such as benzoic and salicylic. Used for determination of the pH of solutions. It is employed in this way for blood, and in media to differentiate typhoid and paratyphoid bacilli, <i>g.v.</i> |
| Phenol Violet | pH 8 yellow through blue to pH 10 violet | 0.15 g. of thymol blue and 0.025 g. of phenolphthalein warmed with 3.25 ml. of N/10 NaOH and 5 ml. of alcohol (90%) until dissolved, and diluted with alcohol (20%) to 250 ml. | Used as an alternative to phenolphthalein in the titration of boric acid. |

| INDICATOR | COLOUR CHANGE | STRENGTH AND SOLVENT | REMARKS |
|--|---|---|---|
| Potassium Chromate | Red colour due to formation of silver chromate, occurring only after halide is all precipitated | 1 in 20 of water. A few drops of the solution are employed | For titrating soluble halides with silver nitrate. The solution of the halide must be neutral, since silver chromate is soluble in acid. |
| Potassium Ferrocyanide | When blue or green coloration is no longer produced | 5% solution in water, to be freshly made. Drops of it or a few small crystals are placed on a white tile | Employed for titrating ferrous iron with potassium dichromate. Also used in titrating phosphate or arsenate with uranium acetate solution. In this case the end-point is the <i>appearance</i> of a brown colour on the ferrocyanide crystal (after boiling the solution) due to formation of uranium ferrocyanide. |
| Rosolic Acid <i>Syn.</i> Corallin, Aurin | pH 6 yellow pH 7 pink pH 8 red | 0.5% in alcohol (50%) | Responds to inorganic bases and to organic bases. Not suitable for use in presence of ammonia or CO ₂ . End-point not very sharp. |
| Starch | Formation or disappearance of blue colour | 0.5% in water, boiled and cooled | For use in the titration of oxidisable substances, e.g., arsenious acid or thio-sulphate, with iodine or <i>vice versa</i> . |
| Thymol Blue (Thymol-sulphone-phthalein) | pH 1.2 red pH 2.8 yellow pH 8 greenish-yellow pH 9.6 blue | 0.1 g. warmed with 4.3 ml. of N/20 NaOH and 5 ml. of alcohol (90%) until dissolved, and diluted with alcohol (20%) to 250 ml. | In the alkaline range it gives a sharper end-point than phenolphthalein and is used in the titration of citric acid. Used also for determination of the pH of solutions. |
| Thymol-phthalein | pH 8 colourless pH 10 blue | 0.04% in alcohol (60%) | Used for determination of the pH of solutions. |
| Turmeric | Orange red with alkalis, yellow with acids | 10 g. macerated with 60 ml. of alcohol (90%) for 7 days and filtered | Responds to inorganic or organic acids and bases. Requires daylight. Not very satisfactory for alkaloïds, except atropine. Suitable for estimating boric acid. Sensitive to ammonia (1 in 35,000) and potash (1 in 180,000). |

Mixed Indicators

By suitably mixing different indicators, mixed indicators can be prepared to have sharp colour changes over selected narrow ranges of pH.

A mixture of equal parts of neutral red and methylene blue changes from violet-blue to green (from acid to alkaline solution) at pH 7. It may be used for the titration of a weak acid with a weak base.

A mixture of α -naphtholphthalein, 1 part, and phenolphthalein, 2 parts, changes from pale rose through green to violet at pH 9.6, and can be used for titrating phosphoric acid to the dibasic stage.

A mixture of thymol blue, 6 parts, and cresol red, 1 part, changes from rose at pH 8.2, through blue at pH 8.3 to violet at pH 8.4, and can be used for titrating carbonates to the bicarbonate stage.

Fluorescent Indicators

Substances whose fluorescence in ultra-violet light changes in intensity or colour with change in hydrogen ion concentration of their solutions have found application as indicators in volumetric analysis. The titration of coloured wines, fruit juices and dark-coloured vinegars can normally only be carried out by largely diluting the samples, when errors are introduced. By using as indicators such substances as quinine, umbelliferone, etc. (see Table), and carrying out the titration in a darkened room, using ultra-violet light, dilution of the samples is not necessary and turbid solutions may be used. The use of fluorescent indicators also eliminates errors introduced by the use of ordinary indicators in the presence of salts and proteins. (See Holthoff, *Z. anal. Chem.*, 1933, 91, 263; S. Malowan, *Chem. Ztg.*, 1933, 37, 824; M. Haitinger, *Mikrochemie*, 1935, 16, 321.)

The following table, from *Fluorescence Analysis in Ultra-Violet Light*, by J. A. Radley and J. Grant, compiled from data in the literature, shows substances suitable as fluorescent indicators in ultra-violet light for various pH ranges.

| INDICATOR | COLOUR CHANGE | pH RANGE |
|---|---|---------------------------|
| Acridine | Green to violet | 4.9 to 5.1 |
| Acridine orange | Colourless to yellow-green | 8.4 to 10.4 |
| Benzoflavine | Yellow to green | 0.3 to 1.7 |
| Coumaric acid | Colourless to green | 7.2 to 9.0 |
| Coumarin | Weak green to bright green | 9.8 to 12.0 |
| Dichlorofluorescein | Colourless to green | 4.0 to 6.0 |
| 3 : 6-Dihydroxyphthalic dinitrile | Blue to green | 5.8 to 8.2 |
| 3 : 6-Dihydroxyphthalimide | Green to yellow-green | 6.0 to 8.0 |
| 3 : 6-Dihydroxyxanthone | Colourless to blue-violet | 5.4 to 7.6 |
| Eosin | Colourless to green | 2.5 to 4.5 |
| Erythrosin | Colourless to green | 4.0 to 4.5 |
| 4-Ethoxyacridone | Green to blue | 1.2 to 3.2 |
| Fluorescein | Colourless to green | 4.0 to 4.5 |
| β -Methyl-umbelliferone | Colourless to blue | 6.5 to 7.5 |
| β -Naphthionic acid | Azure blue to violet | 12.0 to 13.0 |
| β -Naphthol | Blue to colourless | 6.0 to 8.0 |
| β -Naphtholsulphonic acid | Dark blue to bright violet | 9.0 to 10.0 |
| β -Naphthylamine | Colourless to violet | 2.8 to 4.4 |
| Quinine | (i) Blue to violet (ii) Violet to colourless | 5.9 to 6.1 9.5 to 10.0 |
| Salicylic acid | Colourless to dark blue | 2.5 to 3.5 |
| Umbelliferone | Colourless to blue | 6.5 to 7.6 |

The advisability of using a darkened room for titrations using fluorescent indicators causes difficulty in reading the burette. This may be overcome by the method, suggested by Grant (*J. Sci. Instrum.*, 1932, 9, 359), of using a weighted, sealed, hollow tube containing quinine sulphate solution, which floats upright on the surface of the liquid in the burette. The slightest illumination with ultra-violet light causes the float to fluoresce and thus illuminate the meniscus and the graduations of the burette.

Oxidation-Reduction Indicators

These depend for their colour not upon the absolute concentration of a particular ion such as the hydron but upon the ratio of the concentration of two ions, one corresponding to a higher stage of oxidation of the other.

Diphenylamine is not oxidised by permanganate in the presence of ferrocyanide until the ratio ferricyanide/ferrocyanide is greater than a certain value at which practically the whole of the ferrocyanide has been oxidised. In using diphenylamine the ferrocyanide solution is acidified with sulphuric acid until the acidity is slightly more than normal and a few drops of a 1% solution of the indicator in sulphuric acid are added. The completion of the titration is shown by the development of a violet colour.

Diphenylamine may also be used as internal indicator in the titration of ferrous salts with dichromate. The ferrous iron solution is acidified to about N/2 or N/1 with sulphuric or hydrochloric acid, 2 ml. to 3 ml. of phosphoric acid is added together with a few drops of indicator solution. Titration is continued to the production of an intense violet colour.

Diphenylbenzidine may be used in place of diphenylamine in iron titrations. It is also of especial value in the determination of zinc by titration with potassium ferrocyanide. A trace of ferricyanide is added to the ferrocyanide solution used. To the zinc solution add 3 ml. of concentrated sulphuric acid per 100 ml. of solution, 10 g. of ammonium chloride and a few drops of 1% diphenylbenzidine in concentrated sulphuric acid; on adding the ferrocyanide solution a blue-violet colour is obtained which persists until the zinc has been precipitated as $K_2Zn_3[Fe(CN)_6]_2$, when the colour changes sharply to pale green.

2:6-Dichlorophenolindophenol is used in N/1000 solution for the determination of ascorbic acid acidified with 5% trichloroacetic acid or metaphosphoric acid. The titration gives accurate results if carried out rapidly and the reagent acts as its own indicator, giving a red colour. (See also pp. 670, 699 and 753.).

Indigo-Carmine is used in concentrated sulphuric acid to detect the presence of small quantities of oxidising agents such as a nitrate. The blue colour changes to pale yellow on oxidation. It has been used as an indicator for ceric sulphate titrations.

Phenylanthranilic acid 0.1% of 0.3% aqueous solution is used in ceric sulphate titrations, changing from colourless to deep pinkish-violet on oxidation.

Xylene-cyanole FF in 0.1% aqueous solution changes from green to orange-pink in the presence of oxidising agents and is recommended for ceric sulphate titrations (Ferre, *Quart. J. Pharm.*, 1937, 351).

Adsorption Indicators

These are coloured compounds which, at the completion of a titration, are adsorbed upon the precipitate produced in the reaction, forming a complex with a colour distinct from that of the solution.

Fluorescein (1 mg. per litre of the liquid being titrated) may be used in the titration of a chloride with silver nitrate. Completion of the titration is indicated by the formation of a pink colour on the precipitate, the solution being greenish-yellow. The chloride solution should be neutral or faintly acidified with acetic acid and not more dilute than N/100. The indicator may also be used in titrations of thiocyanates with silver nitrate, of sulphates with barium hydroxide, and of neutral oxalates with lead acetate. Sodium hydroxide can be titrated very accurately with lead acetate in the presence of fluorescein, the colour change from greenish-yellow to pink being most conspicuous in concentrations down to N/200.

Dichlorofluorescein (2 drops of 0.1% solution in 60% alcohol per 100 ml.) may be used similarly for chlorides in great dilution and in the presence of acetic acid. It gives a sharp end-point and accurate results in the titration of metaborates, orthoborates and perborates (in solution made neutral with nitric acid) with lead acetate. The most convenient concentrations are N/100 to N/10 and the colour change is greenish-yellow to pink.

Dibromofluorescein (1 mg. per litre of liquid titrated) may be used for the titration of orthophosphates in neutral or dilute acetic acid solution with lead acetate, a red colour being obtained at the end-point.

Eosin (10 mg. per litre) may be used for titrating bromides in very dilute solution (down to N/1000). The titration may be carried out in the presence of N/10 nitric acid or, preferably, N/10 acetic acid. The precipitate acquires a magenta colour, the liquid being red.

Di-iododimethylfluorescein (5 to 10 drops of 1% solution per 100 ml.) is better than eosin for titrating iodides, the colour of the precipitate being blue-red and of the solution orange-red. It may be used in the presence of small amounts of chloride.

Diphenylcarbazine (1 drop of 0.1% alcoholic solution to 25 ml.) may be used for cyanide, the pink colour of the liquid becoming pale violet (almost colourless) on the colloidal precipitate before opalescence is visible.

Alizarin (2 drops of 0.5% alcoholic solution to 25 ml.) may be used in the titration of nitrates with a solution of titanous chloride in hydrochloric acid, the end-point being denoted by the permanent grey-green colour of the precipitate and the disappearance of the original red colour of the solution.

Other adsorption indicators which have been recommended for argentometric determination of halides are erythrosin, tartrazine, phenosafranine, chromotrope F4B, diphenylamine blue, bromophenol blue, and rhodamine 6G.

FLUORESCENCE ANALYSIS IN ULTRA-VIOLET LIGHT

Ultra-violet rays have wavelengths from 136 to 4000 Å and in addition to their penetrative power, have the useful property of being absorbed by certain substances which then emit visible radiations. If the emission lasts during the period of excitation only it is termed fluorescence, as distinct from phosphorescence which persists when the exciting source is removed.

When the fluorescence is characteristic of the substance irradiated, it may be used as a means of analysis. For ordinary work it is sufficient in most cases to note the intensity and colour, but the method is applicable to a greater range of materials if the light emitted is examined spectroscopically. In general, the intensity is proportional to the amount of active substance present and quantitative methods have been devised based on this proportionality.

Qualitative Methods.

- (a) *Direct irradiation.* The substance is exposed to ultra-violet light from a suitable source and the nature, colour and intensity of the fluorescence compared with those from a genuine sample of material. The comparison may be carried out on the solid substance or on solutions; the latter may be prepared with various solvents and may have an acid, alkaline or neutral reaction. A refinement of the method is to observe the fluorescence under a microscope (see J. Grant, *Nature, Lond.*, ii/1934, 635, also M. Servigne, *Ann. Chim. Applic.*, 1937, 17, 313).

- (b) *Chemical reactions.* The suspected substance is treated with a reagent which should produce a fluorescent compound and the appearance of that compound noted. Alternatively, if the substance itself is fluorescent a reagent may be used which destroys the fluorescence of the substance tested.
- (c) *Capillary analysis.* This depends on observation of the nature of the fluorescent zones produced on filter paper impregnated with the substance (*vide infra*).

Quantitative Methods.

- (a) *Trial and error.* A number of mixtures containing known amounts of the fluorescent substance are compared with the sample in ultra-violet light and an approximate match is obtained with one of them.
- (b) *Photometry.* The intensity of the fluorescence, other conditions being equal and over a restricted range of concentrations, is proportional to the amount of fluorescent substance present and may be determined photometrically. Visual instruments and photo-electric instruments are available for this purpose.
- (c) *Volumetric analysis.* A change in the fluorescence of a particular substance on addition of a reagent may be used to determine the substance itself. Quinine, for example, may be titrated in ultra-violet light without the addition of an indicator, the change in fluorescence of the quinine being taken as the end-point.

Source of Ultra-violet Light.

For the production of ultra-violet rays many lamps are available with widely differing electrodes. Most commonly used is a quartz mercury-vapour lamp, such as the *Hanovia Analytic Quartz Lamp*. This consists of an evacuated quartz tube with a small vessel at each end containing mercury, which is in contact with metallic leads ground into the quartz and fixed by cement. The lamps, which are made available for use with direct or alternating current, are suspended so that the floor of the evacuated tube may be tilted by means of a handle in front of the apparatus. The current having been switched on, the mercury makes a short circuit between the two poles on tilting the tube; at first the mercury boils and splutters around the negative pole but as soon as the tube is sufficiently hot and filled with vapour, this ceases and an arc fills the tube. This arc is at first bluish and does not reach its maximum intensity for about five minutes.

For fluorescence analysis the lamp is enclosed in a cabinet in the lower portion of which is a dark chamber in which is placed the substance to be examined. Light from the burner passes into the chamber through a filter of nickel oxide glass which transmits only the rays of suitable wavelength.

The *Callophane* is a simple device for obtaining ultra-violet light, though the intensity of the radiation obtained is much less than that from mercury-vapour lamps. It consists of a folding wooden box, the lid of which is hinged at one end and fitted with

a glass filter transparent only to the ultra-violet rays of daylight. In use, the substance examined is placed in the box which is held against the face in such a way as to exclude all light except that transmitted through the filter. The source of light may be daylight or a high-power electric lamp (e.g., 200 watts).

Caution. Ultra-violet light may produce harmful effects on the eyes, especially in the case of artificial sources where the light is very intense. Care should be taken not to expose the eyes to the light for any length of time.

Technique of Fluorescence Analysis. The container in which the sample is placed should give no fluorescence itself. Black filter papers may be used or Petri dishes covered with cellophane, but quartz vessels are preferable. Since the state of the sample may influence the fluorescence, attention should be given to the fineness of division in the case of a powder, to the solvent and the concentration used in the case of solutions. Samples and authentic specimens must be compared under exactly the same conditions and it is essential to take care that any changes occurring in the colour and intensity of the fluorescence during irradiation do not pass unobserved.

Capillary Analysis. This is due to Danckwortt and Pfau and has been applied to the examination of solutions of drugs, resins, etc.

If a filter paper is held vertically with one edge in a solution, liquid is drawn up by capillary attraction, and when the wet portion is examined under the lamp characteristic zones are obtained from many substances. The test does not give comparable results unless a standard procedure is followed. The paper should be non-fluorescent and should be cut into strips 2 cm. by 30 cm., all strips being cut in the same direction since the absorption may vary according to the grain of the paper. The paper is then clamped by its upper edge so that it hangs vertically, 5 mm. of the lower end being immersed in the solution, and it may be left for as long as 24 hours to ensure that a state of equilibrium has been reached. Temperature, concentration, humidity, air-currents and time must all be carefully controlled if comparable results are desired. The papers obtained are examined under the lamp and, for semi-quantitative work, are compared with those obtained by an identical procedure from samples of known composition and concentration. More accurate quantitative results are obtained by examination of the fluorescences photometrically.

Applications of Fluorescence Analysis

Bacteriology. Examination under ultra-violet light of cultures of bacteria has proved of value in identification. The procedure is to plate out cultures in the usual way and after incubation to transfer the colonies on a platinum wire to a piece of damp white filter paper in a Petri dish. The specimen is then dried in an oven at a low temperature and examined in ultra-violet light. As most Petri dishes and culture media themselves fluoresce this technique is necessary in order to obtain the fluorescence of the colonies free from interference by other fluorescent bodies. Cultures for comparison should be of the same age and should have been grown on the same medium. The Petri dishes may conveniently be covered with transparent cellulose tissue, which is transparent

to ultra-violet light. For example, various types of paratyphoid organisms and the Flexner and Shiga types of dysentery bacilli have given different fluorescence colours by which they may be distinguished.

Alkaloids. The following alkaloidal substances show a more or less pronounced fluorescence which disappears when they are quickly removed from the influence of the rays.

| | |
|-------------------------------|-------------------------|
| Aconitine | distinct light blue |
| Apomorphine | deep blue |
| Atropine sulphate | faint bluish |
| Berberine | distinct yellow |
| Berberine hydrochloride | distinct yellow-green |
| Cinchonine | light bluish |
| Cinchonine sulphate | distinct clear white |
| Codeine | light clear yellow |
| Colchicine | distinct yellow-green |
| Emetine | distinct yellow-red |
| Hydrastine | distinct light green |
| Morphine | pronounced light blue |
| Narceine | pronounced yellow-green |
| Narcotine | light greenish |
| Papaverine | pale light yellowish |
| Pilocarpine | white |
| Piperine | faint bluish |
| Quinine hydrochloride | pronounced light blue |
| Quinine sulphate | pronounced light blue |
| Solanine | pronounced light yellow |
| Thebaine | reddish yellow |
| Veratrine sulphate | marked light blue |
| Yohimbine hydrochloride | deep yellow-green |

Danckwört and Pfau (*Analyst*, 1927, 707), using the method of capillary analysis, have examined *extracts and tinctures* containing alkaloidal constituents. Three extracts of each drug are employed, one neutral, one acid and one ammoniacal. As examples may be mentioned the following:—

| | IN NEUTRAL AND ACID SOLUTIONS | IN ALKALINE SOLUTION |
|---------------------|--|--|
| Cinchona .. | Upper zone deep blue, lower strip red-violet. | No blue zone, no red-violet colouring. |
| Belladonna | Upper zone broad yellow-green, underneath light bluish. | Dirty green, below red-brown, underneath no bluish shade. |
| Digitalis .. | Above narrow blue-green zone, then green-brown. | No upper zone, deep dirty brown. |
| Hyoscyamus | Upper zone light blue-green, dirty green, then bright. | Blue-green, underneath dirty green-brown. |
| Stramonium | Faint narrow pale yellow zone. | Similar to the acid solution. |
| Opium .. | Upper zone distinct light blue, underneath two yellowish zones. | Light blue, underneath several zones. |
| Ipecacuanha | Dark blue, underneath light blue. | No blue zones, faint light yellowish. |
| Hydrastis .. | Upper zone dark blue, the other strip deep yellow. | Faint bluish wider zone, underneath deep yellow. |
| Areca .. | Upper zone distinct blue, underneath coffee-brown. | Fainter blue, underneath green-brown. |
| Colchicum .. | Broad light yellow zone, underneath colourless. | Yellow zone fainter. |
| Strophanthus | Narrow bluish zone, underneath darker zone, then lighter. | Wider lighter zone, yellow-brown, then light. |
| Nux Vomica | Faint bluish, underneath at first dark green, then blue. | Light bluish, underneath no green zone. |
| Veratrum .. | Dirty yellow-brown, underneath light greenish, then violet-blue. | Above faint light-bluish wider brown zone, then light green. later bluish. |

Powdered Drugs. Some broken or powdered vegetable drugs give a fluorescence which is of value for their detection in mixtures and when present as adulterants in other drugs. The freshly broken surface of *hydrastis* gives a characteristic bright yellow fluorescence, the scraped or broken surface of *gelsemium* gives a marked blue colour. *Gentian* powder shines with a white or light blue colour and adulteration with *rumex* powder is detectable by the golden yellow or dark green fluorescence of the latter. American *viburnum prunifolium* and Russian *viburnum opulus* are distinguished by the reddish fluorescence of the former and the bright green fluorescence of the latter. *Rhubarb* gives a reddish-brown fluorescence and the presence of rhapontic rhubarb can be detected by the bright violet or lilac colour which it gives in ultra-violet light.

By extraction of the rhubarb with alcohol and examination of the solution by capillary analysis quantitative examination of rhubarb for rhapontic rhubarb is possible.—T. E. Wallis and E. R. Withell, *Quart. J. Pharm.*, 1934, 574. (See also S. K. Crews, *ibid.* 1936, 434.)

An ether extract of *cannabis indica* gives a green fluorescence due to the presence of cannabinol and this test is said to be more reliable than the Beam test which is not specific.—J. Khouri, *Ann. Med. Legale*, 1936, 16, 249.

References. P. Kampf, *Pharm. Acta Helvet.*, 1933, 8, 170, 214 (Detection of adulteration of drugs by capillary analysis). G. Fodor and A. Kichler, *Pharm. Monatsh.*, 1930, 11, 29 (Examination of galenicals in ultra-violet light). L. Rosenthaler, *Pharm. Ztg. Berl.*, 1933, 1063 (Examination of galenicals). M. Haitinger, *Mikrochemie*, 1935, 16, 353. (Detection of alkaloids by fluorescence microscopy). G. E. Trease, *Textbook of Pharmacognosy* (Various pharmacognostical applications).

Peppermint Oils. Small but distinct differences in fluorescence are observable between English, American and Japanese peppermint oils, but the determination of mixtures by direct observation is unsatisfactory. It is shown that the sensitivity of the test of the B.P. 1932 for differentiating between English and Japanese peppermint oils (the copper-coloured fluorescence developed after heating with nitric-acetic acid mixture) is greater in ultra-violet light than in daylight.—J. A. Radley, *Chem. & Drugg.*, ii/1933, 768.

Diethyl Phthalate. The fluorescence in ultra-violet light of diethyl-phthalate (used for denaturing industrial methylated spirit) is used by the American Customs authorities to control the distribution of spirit for special purposes.—G. Rattray and G. R. Milne, *Pharm. J.*, i/1934, 393.

Phenolphthalein. The detection of phenolphthalein when present in preparations containing also polyhydroxy anthraquinone derivatives which also give reddish colours in alkaline solution (such as aloes, cascara, rhubarb, senna) is possible by treatment of a potassium hydroxide solution of the mixture with hydrogen peroxide and condensation of the resulting phthalic acid with resorcinol by heating. Fluorescein so formed can be detected with the aid of ultra-violet light in amounts representing 0.01 milligramme or more of phenolphthalein.—E. H. Maechlin, *Industr. Engng Chem. (anal. Edn.)*, 1938, 50, 536.

Chalk. Fluorescence under ultra-violet light has been used to distinguish between precipitated and prepared chalk in alkaline stomach powders.—W. E. Naylor and A. Surfleet, *Pharm. J.*, 1936, 261.

Oestrogens. As little as 0.00025 milligrams of oestrone can be detected by the greenish-yellow fluorescence in ultra-violet light given by chloroform solution of the product of coupling with phthalic anhydride on heating with stannous chloride.—I. S. Kleiner, *J. biol. Chem.*, 1941, 138, 783.

Soaps. Forensic applications of the fluorescence of commercial soaps and of soap stains in fabrics.—M. W. Partridge, *Quart. J. Pharm.*, 1942, 2, 21.

Olive Oil. The fluorescence of olive oil in ultra-violet light has been shown to be of value for the detection of refined oil in the presence of virgin oil. The yellow fluorescence of a virgin oil is changed to a bluish colour by oxidation or strong heating and this is said to account for the bluish colours given by refined oils. (See Lunde and Stiebel, *Fluoreszenz-Messungen an Olivenölen.*)

Occasionally substances giving a dark purple or brown masking fluorescence are found added to adulterated virgin oils (e.g., containing tea-seed or refined olive oils) which give similar analytical figures to genuine virgin oil except for the iodine value of the unsaponifiable matter (Bolton-Williams number), which is lowered. It has been found that this masking fluorescence may be removed by shaking the oil with decolorising charcoal and examining after filtration.

The presence of a bright blue fluorescence should render the oil suspect.—T. T. Cocking and S. K. Crews, *Quart. J. Pharm.*, 1934, 531.

Although examination of oils by ultra-violet light does not necessarily lead to identification of adulteration, it can be very valuable in indicating the origin and history of the sample, especially on comparison with specimens of known history and purity (see Bureau of Standards, *J. Franklin Inst.*, 1927, 204, 805; also E. R. Bolton and K. A. Williams, *Analyst*, 1933, 84, for detection of fuel oil in whale oil).

Sterols and Vitamins. Among reactions for cholesterol and its related compounds, the following are rendered more sensitive if examined by ultra-violet light:—A red colour and a green fluorescence is obtained if a solution of cholesterol in glacial acetic acid is warmed for 5 minutes with acetyl chloride and zinc chloride (sensitivity 1 in 80,000).

Bruckner's test for ergosterol:—The sample is dissolved in 2 ml. of benzene, and 1 ml. of acetic anhydride, 0.5 ml. of acetone, 0.5 g. of anhydrous zinc chloride and a crystal of copper acetate are added.

A blue-violet colour and a red fluorescence in ultra-violet light are obtained in the presence of 0.001 mg. per ml.—J. Bruckner, *Biochem. Z.*, 1934, 270, 346.

Determination of Vitamin A. By dividing a beam of filtered ultra-violet light of wavelength 328 μ into two portions in the vitameter, allowing one portion only to pass through the solution of vitamin (e.g., liver oils), then focussing both portions on a screen fluorescing under the action of ultra-violet light of 328 μ and comparing the intensity of the two portions, a measure is obtained of the vitamin A present since one half will be less intense due to the absorption of the light by the vitamin.

The fluorescence of butters, spinach, tomatoes and other foodstuffs has been correlated with the vitamin A content. (J. W. Woodrow and A. R. Schmidt, *Physiol. Rev.*, 1933, 43, 379.)

Determination of Aneurine. The vitamin is oxidised with alkaline potassium ferricyanide and the solution extracted with secondary butyl alcohol. The quantitative examination of the bluish-violet fluorescence in ultra-violet light, by allowing the transmitted light to pass to a photo-selenium cell and comparing the current produced with that given by a standard solution of quinine sulphate, is a method used commercially for determining aneurine. The method is based on the work of Cohen (*Acta brev. neerl. Physiol.*, 1934, 46) who determined lactoflavin by a similar method. (See also Peters, *Nature, Lond.*, 1/1935, 107; also *Analyst*, 1942, 67, 15.)

Photo-electric fluorimeters have become available and in particular have been used for the determination of aneurine, the method consisting in measuring the changes in solvent and test solution by addition of equal quantities of aneurine to each (see Hills, *Biochem. J.*, 1939, 33, 1966, and Booth, *J. Soc. chem. Ind.*, 1940, 59, 181).

Suitable photo-electric fluorimeters are described by R. H. Müller, *Industr. Engng Chem. (anal. Edn.)*, 1941, 13, 714. The Spekker photo-electric fluorimeter is described by G. F. Lothian (*J. Sci. Instr.*, 1941, 18, 200) and the effect of impurities, of fluorescing impurities and of wavelength are discussed by this author (*J. Soc. chem. Ind.*, 1942, 61, 58).

Preservatives in Foodstuffs. Sodium salicylate is detected by its violet fluorescence in ultra-violet light, the detection of 1 part in 25,000 in milk being possible.—J. Volmar, *J. Pharm. Chim., Paris*, 1/1927, 435.

Sulphur dioxide may be detected when 0.25 mg. is present by the following method:—To the sample in a boiling tube add 10 ml. of water, a little hydrochloric acid and boil gently, distilling over about 3 ml. into a tube containing 5 ml. of water, 1 ml. of pure 40 vol. hydrogen peroxide and 0.5 ml. of a saturated solution of quinine alkaloid in water. Any SO_2 is oxidised to H_2SO_4 which causes the quinine to fluoresce in ultra-violet light.—J. Grant and J. H. W. Booth, *Analyst*, 1932, 514.

Dulcin in the Presence of Saccharin. One per cent. of dulcin in the presence of saccharin may be detected by the violet fluorescence in ultra-violet light developed after ten minutes when 10 mg. of such a mixture is heated with 10 mg. of resorcinol and 2 ml. of sulphuric acid for two minutes, cooled and made alkaline with sodium hydroxide. If ammonia is used in place of sodium hydroxide a red-brown fluorescence is obtained immediately.—H. J. Vlezenbeek, *Pharm. Weekblad.*, 1937, 74, 127.

Biological Use. The examination of body fluids in ultra-violet light has provided useful information on the fate of drugs. The method has been used

to detect drugs passing into human milk and amniotic liquid—cases in which chemical examination would be difficult.—R. Joachimovits, *Mschr. Geburtsh. Gynak.*, 1929, 83, 42; see also N. von Jancsó, *Klin. Wschr.*, 1932, 689, and E. Singer, *Science*, 1932, 289. See A. Hadjioloff (*Compt. rend. Soc. Biol.*, 1938, 128, 1096) for fluorescence analysis of tissue cultures.

Organic Substances. Many organic substances exhibit characteristic fluorescences in ultra-violet light and examination of substances by this means, especially when comparison is made with authentic specimens, has given information concerning their constitution or identity. Thus the examination of the spectra of the fluorescences obtained in ultra-violet light have enabled Cook and his co-workers to follow the isolation of the carcinogenic constituents of coal tar and pitch, the active hydrocarbon 1 : 2-benzpyrene being obtained by means of its picrate.—J. W. Cook, *J. chem. Soc., Lond.*, 1933, 395.

Technical Uses. Fluorescence analysis in ultra-violet light is also applied to the examination of dyestuffs, textiles, papers, leathers, paints and varnishes, cellulose materials, finger prints, bloodstains, rubber products, gems, documents and museum specimens.

Other references.—J. A. Radley and J. Grant, *Fluorescence Analysis in Ultra-Violet Light*, 3rd Edn., 1939, London; J. Grant, *Fluorescence Analysis as an Aid to Chemical Analysis*, *Current Sci.*, 1936, 4, 801; H. Neugebauer, *Die Kapillar-Lumineszenz-analyse im pharmazeutischen Laboratorium*, 1933, W. Schwabe, Leipzig; P. W. Danckwortt, *Lumineszenz-analyse in Filtrierten ultra-violetten Licht*, 1934, Leipzig; H. Fischer, *Die physikalische Chemie in der gerichtlichen Medizin und der Toxikologie mit spezielle Berücksichtigung der Spectrographie und der Fluoreszenzmethoden*, 1925, A. Rudolf, Zurich.

CHROMATOGRAPHIC ANALYSIS

Chromatographic adsorption analysis, the simultaneous adsorption and separation of mixtures of chemical compounds in solution, is becoming of increasing importance owing to its very wide applicability to chemical problems. By stirring charcoal or other adsorbent into a solution of a mixture of substances, the constituents of the mixture may be more or less completely separated into two fractions, a more and a less adsorbable one. The process of separation by adsorption is greatly improved in chromatographic methods which utilise the fact that compounds, often very closely resembling one another in chemical structure, may be adsorbed by a reagent to different extents. Differential adsorption is effected in a chromatogram by allowing the solution to drop through a vertical column of the adsorbent. On washing the column with pure solvent (development of the chromatogram) the adsorbed mixture undergoes innumerable elutions and readsorptions from particle to particle, and as a result the constituents of the mixture move down the column at different rates, so that well defined zones are obtained of the different constituents. These zones can be separated mechanically and the adsorbed substances obtained in solution by washing the zones with suitable solvents.

Chromatographic analysis, which may be employed as a micro- or macro-method, is of value in identifying materials, in concentrating materials, in establishing the homogeneity of materials, in assaying the quality and genuineness of technical products and in purifying products. It has also been applied to the resolution of racemic mixtures into their optically active components. Although

its scope for inorganic chemical work has not yet been sufficiently explored it has been used as an aid to the separation of mixtures in analysis and for partial separation of isotopes.

The method was first used by Tswett in 1906 in separating the constituents of chlorophyll but has been little used since that time until the last few years, when it has been used with remarkable success with most diverse compounds. The method was revived when α - and β -carotene were separated from carrot carotene in light petroleum by adsorption on a column of calcium carbonate. The optically inactive β -carotene, being more strongly adsorbed than the optically active α -carotene, remained as a zone above the latter when the chromatogram was developed by washing with light petroleum (R. Kuhn and E. Lederer, *Ber. dtsch. chem. Ges.*, 1931, 1349). This successful separation led to the increasing use of chromatographic methods in separation of carotenoid substances from vegetable and animal substances. The elegance of the method, the quantitative recovery of all material and the fact that the adsorption can be carried out rapidly at room temperature and in the absence of air, if required, make it a valuable analytical method for the biochemist and analyst, and a method of great importance to the organic chemist.

Apparatus. Although many modifications are possible the essential apparatus consists of a long tube drawn out at one end and mounted in a filter flask. The shape of such a tube makes it impossible to extrude the column unless the glass is cut, but since the individual layers can be washed down gradually, the filter flask can be changed at appropriate times. For extrusion of the column and separation of the zones a more convenient apparatus consists of a long, thin, glass cylinder, covered at its lower end by a removable gauze or bearing a suitably perforated stopper, fitted through a bung into a wider glass cylinder drawn out at its lower end for attachment through a bung to a filter flask. In such an arrangement the lower end of the inner glass cylinder covered by gauze or stoppered, rests on a perforated porcelain disc or gauze introduced into the outer cylinder. Suitable apparatus with ground glass joints is available.

The tubes used may range in size from those suitable for micro-work, as small as 2 mm. diameter by 3 cm. in length to those for large scale work of 10 cm. diameter by 100 cm. in length. Commonly preferred are tubes of 1 to 5 cm. diameter and 20 to 30 cm. in length. For large scale work it is better to use several columns rather than attempt to work with one very large column, due to the difficulty of efficient packing of large columns.

Procedure. The essential condition for successful chromatographic analysis is the careful and uniform packing of the adsorbent into the tube. A suitable support is first provided for the column in the form of a cotton wool plug, or a perforated porcelain or a sintered glass disc. Portions of the adsorbent are added and well tapped down with the aid of a flattened glass rod.

The adsorbent should be of uniform grain size and standardised products are commercially available having that virtue. The upper fourth of the tube should be free for introduction of solution. An alternative method of filling the tube consists in pouring in small quantities of a cream of adsorbent with organic solvent, removing the solvent by slight suction, pressing down the solid remaining with a flattened glass rod and repeating the procedure until the column is obtained of sufficient length. No cracks should appear in forming the column.

Throughout the analysis the column should be kept covered by a layer of solvent so that the upper layers are not disturbed by the dropping liquid. The solution to be examined is drawn through the column by means of a siphon or more usually dropped from a dropping funnel. In the ideal case the solute is adsorbed and the constituents of the mixture are concentrated in a zone at the top of the column. When more pure solvent is filtered through the column (development) the constituents become separated into localised zones at depths dependent upon their affinity for the adsorbent. Slight suction is usually used to facilitate the passage of solvent through the column. With coloured compounds the zones are readily detectable and mechanical separation of the zones after extrusion of the column is normally used, the separate portions being boiled with solvents to remove adsorbed materials. With colourless materials especially if a quartz tube is used, ultra-violet light is of assistance in separating the zones. Alternatively, indicators or chemical reactions may assist in detecting zones, but commonly an empirical procedure is used, fractions of eluted material being examined by physical (optical activity, absorption spectrum or fluorescence) or chemical tests for detection of the separated constituents.

Adsorbents. The following adsorbents have been used: aluminium oxide, calcium carbonate, calcium hydroxide, magnesium oxide, lactose, sucrose, quartz and fuller's earth. The most widely used adsorbent is aluminium oxide which is activated by heating and is of uniform particle size, a suitable grade being one which is retained between 100 and 120 B.S. sieves. Activated alumina of uniform grain size is commercially available, "Mayfair A" Brand and Birlec Activated Alumina being generally considered equal in value to the original product standardised according to Brockmann.

Solvents and Eluents. Usually an adsorbate adheres to any one adsorbent more firmly in a less polar solvent. Adsorption is frequently carried out from light petroleum solution, a single dense zone being obtained which develops only slowly on washing with more light petroleum but more rapidly on passing to benzene, carbon disulphide, chloroform, ethyl acetate, alcohol, acetone, acetic acid, and diethylamine or pyridine. Mixtures of these solvents may be used in succession. In general a solvent in the above list is more effective as an eluting medium than one

preceding it so that alcohol or a mixture of alcohol and benzene or alcohol and light petroleum will often wash the adsorbed constituents quickly through the column. The choice of solvent must, of course, be governed by solubility considerations.

A method particularly suitable for making rapid preliminary analytical surveys and for aiding in the selection of the most suitable solvents, adsorbents and eluents has been described by M. O. L. Crowe (*Industr. Engng Chem. (anal. Edn.)*, 1941, 13, 845). About a quarter of a teaspoonful of each adsorbent is made into a cream with one of the solvents and placed in a cup-shaped depression in a white tile; one drop of the solution to be tested is allowed to flow on to the mixture and the best combination of solvent and adsorbent may thus be selected. A glass Petri dish is one-quarter filled with the chosen dry adsorbent, and then shaken in a tilted position so that the powder settles in the form of a wedge which is 3 millimetres deep at the thicker end. The sample is pipetted into the centre of the tilted dish, so that it flows slowly towards the thin edge of the wedge and at the same time filters downwards into the powder. If the solvent is then added, a drop at a time, broad zones of separated material form; these are very convenient for examination by the fluorescence method.

Applications. Academic applications of chromatographic adsorption are almost unlimited ranging from the mere purification of slightly impure substances to the separation of complex mixtures such as polyenes and carotenoids. In general there is an increase in the tenacity of adsorption on alumina with increase in the number of conjugated double bonds as shown in the diphenyl polyenes and among carotenoids. In the latter group especially, adsorptive behaviour may be correlated with structural features such as extent of conjugations and numbers of hydroxyl groups.

Closely related chemical substances may readily be separated, such as *o*-, *m*- and *p*-nitranilines; *o*-, *m*- and *p*-nitrophenols; geraniol and limonene.—A. Winterstein and G. Stein, *Z. physiol. Chem.*, 1933, 220, 247.

Among the sterols and steroids chromatographic adsorption has found very wide application for example in the enrichment of ergosterol fractions from cholesterol (Winterstein and Stein, *Z. physiol. Chem.*, 1933, 220, 263) for the separation of sterols, particularly stigmasterol, from crude soya bean oil (H. R. Kraybill, M. H. Thornton and K. E. Eldridge, *Industr. Engng Chem. (Industr. Edn.)*, 1940, 32, 1138), for the isolation and separation of constituents of the adrenal cortex (Steiger and Reichstein, *Helv. chim. Acta*, 1938, 21, 828), for the separation of sarsosapogenins (F. A. Askew, S. N. Farmer and G. A. R. Kon, *J. Chem. Soc., Lond.*, 1936, 1399). For the isolation of sapogenins in a purified crystalline form in good yields, chromatographic analysis appears to be the only practicable method and has proved successful on a commercial scale when all normal methods such as attempted crystallisation from solvents, sublimation, and distillation in high vacua have failed to produce purified products. Chromatographic analysis has proved superior to normal fractional crystallisation methods in purifying intermediates in the production of many steroid hormones.

Chromatographic analysis has proved of value in separation, detection, and determination of vitamins. Thus, vitamin A may be detected in the presence of other carotenoids from which it may be separated by adsorption on an alumina column (H. Willstaedt and T. K. With, *Z. physiol. Chem.*, 1938, 253, 40; R. Kuhn and C. J. O. R. Morris, *Ber. dtsh. chem. Ges.*, 1937, 70, 853). Analytically pure vitamin A has been isolated from the unsaponifiable fraction of halibut liver oil by adsorption from petroleum solution on an alumina column, elution with methyl alcohol and repetition of the process using calcium hydroxide as adsorbent. (P. Karrer and R. Morf, *Helv. chim. Acta*, 1933, 16, 625, also P. Karrer, O. Walker, K. Schopp and R. Morf, *Nature*, 1933, 132, 26, and H. N. Holmes, H. Cassidy, R. S. Manly and E. R. Hartzler, *J. Amer. chem. Soc.*, 1935, 57, 1990.)

The concentration of aneurine from extracts of rice polishings, brewer's yeast or wheat germ may be effected by filtration through artificial zeolite. (L. R. Cerecedo *et al.*, *J. Amer. chem. Soc.*, 1937, 59, 1617, 1619, 1621.) Adsorption from ether solution by a column of calcium hydroxide of the product of coupling of aneurine with diazotised 2 : 4-dichloroaniline has been applied to the determination of aneurine (H. Willstaedt, *Naturwissenschaften*, 1937, 25, 682, and H. Willstaedt and F. Barany, *Enzymologia*, 1938, 2, 316).

Vitamin D₂ may be isolated from irradiated 7-dehydro-cholesterol (A. Windaus *et al.*, *Z. physiol. Chem.*, 1936, 241, 100), the method depending on purification of the 3-5-dinitrobenzoate by chromatographing on alumina. Chromatography on alumina has been of value in the preparation of concentrates of vitamin E (Drummond *et al.*, *Biochem. J.*, 1935, 29, 456, 2510), also to the isolation of α -tocopherol (P. Karrer and H. Saloman, *Helv. chim. Acta.*, 1938, 21, 514) and of β -tocopherol (A. R. Todd, F. Bergel and T. S. Work, *Biochem. J.*, 1937, 31, 2257) and forms the basis of a commercial method of separation of α - and β -tocopherols (*I.G. Farbenindustrie, D.R.P.*, 1937, 651474).

Adrenaline is quantitatively adsorbed from solutions at pH 7.2 by a mixture of asbestos and kaolin (H. Fink, *Ber. dtsh. chem. Ges.*, 1937, 70, 1477) and this observation has been made the basis of a method of determination of adrenaline in blood (J. C. Whitehorn, *J. biol. Chem.*, 1934, 108, 633).

The chromatographic method can be used for isolating cozymase from yeast (H. V. Euler *et al.*, *Z. physiol. Chem.*, 1936, 238, 233, and 1937, 246, 64). The method is applicable when a high degree of purity is required and on a relatively large scale.

Technical applications of chromatographic analysis are exemplified by: the characterisation of commercial oils, the detection of added colouring matters in butter, oils and fats (H. A. Bockennoogen, *Rec. Trav. Chim.*, 1937, 56, 351, H. Thaler, *Z. Untersuch. Lebensmitt.*, 1938, 75, 130), the detection of fuel oil in whale oil (E. R. Bolton and K. A. Williams, *Analyst*, 1938, 63, 84), the detection of added pigments in wines (H. Mohler, and W. Hammerle, *Z. Untersuch. Lebensmitt.*, 1933, 70, 193; 1936, 71, 186), the detection of heterogeneity in dyestuffs (R. Frank, *Arch. Pha m. Berl.*, 1937, 275, 125), the detection of adulteration of vegetable drugs such as saffron (R. Franck, *Arch. Pharm. Berl.*, 1937, 275, 125; M. Fichter, *Pharm. Acta Helvet.*, 1939, 14, 158, see *Quart. J. Pharm.*, 1940, 84) and the resolution of bases previously believed to be homogeneous (*D.R.P.*, 627027).

Among interesting and valuable pharmaceutical and analytical applications are the use of chromatographic analysis to the simplification of the determination of cantharidin in tinctures (Valentin and Franck, *Pharm. Ztg. Berl.*, 1936, 943), of caffeine in coffee (Valentin, *Pharm. Ztg. Berl.*, 1937, 527) of the determination of alkaloids in tinctures and extracts of belladonna, cinchona and ipecacuanha (K. W. Merz and R. Franck, *Arch. Pharm., Berl.*, 1937, 82, 345).

Owing to the very fine dependence of adsorption on fine chemical structure and its independence of the method of separation of molecular size considerable advances in chemical knowledge are to be expected, due to the aid of chromatographic analysis. Its application to further specific problems must necessarily follow with increasing exploration. Pharmaceutical applications of chromatographic analysis have as yet, been insufficiently explored. The possibilities of its application in establishing authenticity of galenicals by comparison with standard preparations, in establishing homogeneity in fixed oils, in detection of added synthetic substances to natural essential oils, in detecting adulteration in powdered drugs, in the analysis of complex dispensed mixtures, and in the examination of colouring materials used in medicinal preparations are examples of the opportunities of extending technological applications of chromatographic analysis.

Other References. Zechmeister and Cholnoky, *Die Chromatographische Adsorptionsmethode*, 1938, translated into English as *Principles and Practice of Chromatography*, A. L. Bacharach and F. A. Robinson, London, 1941; *Chromatographic Analysis*, A. H. Cook, Institute of Chemistry, London, Lecture, 1941; *Chromatographic Analysis*, H. H. Strain, 1942, New York.

MICROCHEMICAL ANALYSIS

The use of micro-methods for the analysis of chemical compounds has developed almost entirely during the past twenty-five years and these methods are being increasingly used. The rapid advances, for example, in vitamin chemistry are due in large measure to the employment of micro-methods both in manipulation and analysis. The labours of Emich, Kuhlmann and Pregl

have led to the development of methods of technique which enable micro-methods of analysis to yield more accurate results than the older macro-methods. Many of Pregl's methods involve chemical principles similar to those of the corresponding macro-methods, as in the determination of nitrogen by the Dumas or Kjeldahl methods, of carbon and hydrogen by the combustion method, and of methoxyl by distillation with hydriodic acid. In other instances entirely new methods have been introduced, as in the determination of sulphur and of the halogens.

The prominent feature of Pregl's methods is that each has been tested under the most stringent conditions and with careful observance of detail. For example, in the determination of carbon and hydrogen Pregl examined with infinite patience such effects as the absorption and subsequent liberation of carbon dioxide by rubber tubing, the pressure and speed relations necessary for complete oxidation of the organic material, the equivalence in drying power of the absorption train and drying apparatus, the avoidance of loss of carbon dioxide and water at the rubber connections and the preparation of a tube-filling suitable for the analysis of any type of organic compound. In the Dumas method for nitrogen the dissociation of carbon dioxide into carbon monoxide and oxygen in the presence of metallic copper or copper oxide at high temperatures was carefully studied and the volume error introduced by the adhesion of potassium hydroxide solution to the walls of the azotometer determined. As a result of this and other rigorous examinations of all the factors concerned in an estimation, the methods can now be said to be standardised.

Synopsis of the More Important Methods Used.

For fuller details and for an account of investigations which have led up to the development of the technique adopted, reference should be made to "Quantitative Organic Micro-Analysis of Fritz Pregl," by H. Roth, translated by E. B. Daw, 3rd Edition (London: J. and A. Churchill Ltd., 1937).

Microchemical Balances.

The Kuhlmann microchemical balance, with a maximum load of 20 g., has a constant sensitivity whether loaded or unloaded, a property obtained by having the three knife-edges perfectly straight, lying in one plane and parallel to one another, and further, by the rigid construction of the beam which shows no detectable distortion even at the maximum load. An instrument of the Kuhlmann design yields an accuracy of ± 0.001 mg. on a weight of 20 g. The instrument has a rider adjustment carrying a lens. The rider scale consists of 100 uniform notches, cut by a dividing engine in such a manner that the rider is forced into the lowest point of the notch. The rider weighs 5 mg. The balance is so constructed that it is in equilibrium when unloaded and the 5 mg. rider is in the first notch on the left over the left pan suspension. A displacement of the rider to the one-hundredth notch which is over the right-hand pan suspension corresponds to a load of 10 mg. in the right pan. A rider displacement of one notch, therefore, corresponds to a change in load of 0.1 mg. on the right pan of the balance. This corresponds to an alteration of the equilibrium position of ten divisions on the pointer scale which is magnified by use of a mirror or lens. Thus one division of the pointer scale corresponds to 0.01 mg. and as the oscillations of the swinging pointer can be read to one-tenth of a division after

a little practice, it follows that if all conditions are carefully observed one can weigh with an accuracy of ± 0.001 mg. = $\pm 1\gamma$.

Counterpoises should be prepared for objects which are weighed repeatedly, such as boats, platinum crucibles, filter tubes, absorption apparatus, etc. In setting up the balance care should be taken that there is freedom from vibration and from temperature changes such as might be caused by nearby heating or lighting units. It is important that the temperature of the balance room and the combustion room should be the same.

Tests for Organic Compounds. Lassaigne's sodium fusion test is unsatisfactory. Middleton's method (fusion with sucrose and sodium carbonate or zinc and sodium carbonate) with modifications can be applied on very small quantities of material.—C. L. Wilson, *Analyst*, 1938, 332.

Determination of Carbon and Hydrogen. Solids are weighed in boats of platinum, porcelain or resistance glass, and liquids are weighed in capillary tubes. About 3 to 5 mg. of substance is used for an analysis. The oxygen used must be free from carbon dioxide and organic constituents. It is drawn through the apparatus by means of slight suction by a head of water in a Mariotte flask attached by means of a calcium chloride guard tube to the absorption train and the combustion tube. It passes through a pressure regulator and a bubble counter containing a little 50% potassium hydroxide solution, then through a U-tube, containing in one limb calcium chloride and in the other soda lime, into the combustion tube, the rate of bubbling being 4 ml. per minute. The combustion tube of hard glass has an external diameter of 9.5 to 10.5 mm. and is 500 mm. long exclusive of a neck into which the combustion tube passes, the neck being 23 to 25 mm. in length and 2 to 2.5 mm. in diameter. The calcium chloride absorption tube is attached to this neck. From the neck the filling of the combustion tube consists, in order, of ignited asbestos, lead peroxide, a loose asbestos layer, silver wool, asbestos, lead chromate and copper oxide mixture, and finally a stopper of silver wool to keep the filling in place. Between the filling and the wide end of the tube, which is attached to the U-tube and bubbler, is placed the boat containing the substance to be analysed. In order to obtain correct hydrogen values it is therefore necessary to maintain the lead peroxide at a constant temperature not only during the combustion but also when pre-heating the tube. This is done by means of a heating mortar in which decahydronaphthalene (Dekaline) is boiled, the tube around the lead peroxide layer being thus surrounded by boiling decahydronaphthalene. The absorption train, consisting of calcium chloride tube and soda lime tube, is attached to the neck of the combustion tube, and the soda lime tube via a guard tube of calcium chloride to the Mariotte flask. Because of its tendency to absorb some carbon dioxide, the calcium chloride tube must first be "saturated" with carbon dioxide, and the gas removed by drawing dry air through whilst the tube is attached to the apparatus. The calcium chloride and soda lime tubes, which are both of specified dimensions, are then carefully wiped in consistent manner and weighed before and after the experiment to 0.01 mg.

Before combustion of the substance, the combustion tube is filled with oxygen, while the lead peroxide is heated to the required temperature and the remainder of the combustion tube filling heated to 550°. The absorption train and Mariotte flask are then attached, the boat and substance introduced and all connections carefully made. A short roll of wire gauze is placed round the tube over the portion containing the boat and sample and the combustion carried out by heating carefully, applying heat from a bunsen burner from the wide end of the combustion tube and moving the burner gradually towards the boat as the combustion proceeds. The time required for combustion is 45 minutes. The absorption train is then disconnected, the calcium chloride and soda lime tubes separated, carefully wiped as before, placed in the balance after five minutes and weighed after a further five minutes. After weighing, another analysis may be carried out immediately.

An improved apparatus involving electrical heating, a new type of manometer and a special filling consisting of lead and cerium oxides and silver salts, which absorbs quantitatively halogens, sulphur and arsenic and enables combustions to be carried out more rapidly than formerly is described by G. Ingram, *J. Soc. chem. Ind., Lond.*, 1942, 61, 112.

By careful attention to details given by Pregl and other workers, results are obtainable of accuracy $\pm 0.3\%$ for carbon and also for hydrogen. The results obtainable are more accurate than those obtained by macro-methods. The

method is applicable to organic compounds of most varied structure. When applied to the analysis of metallic organic compounds, such as those containing silver, gold, or platinum, the residue of metal in the boat can be weighed and the metal determined. Organic salts of iron, chromium, aluminium, copper and tin give good results for the metals, the residue in the boat being weighed as oxide (Fe_2O_3 , Cr_2O_3 , Al_2O_3 , CuO or SnO_2) after being heated strongly in the current of oxygen. Magnesium and lead can similarly be weighed as oxide (MgO and PbO) if the heating is not carried out too strongly.

The Determination of Nitrogen (Micro-Dumas). The principles of the micro-Dumas method are similar to those of the macro method. Carbon dioxide, prepared from air-free marble and hydrochloric acid in a Kipp generator, passes over the heated sample mixed with copper oxide in a platinum or porcelain boat in the combustion tube. The products of the combustion pass successively over copper oxide, reduced copper (to reduce oxides of nitrogen to nitrogen), and finally over more copper oxide. The gases then enter the micro-azotometer which is filled with 50% potassium hydroxide solution containing barium hydroxide to prevent frothing. The air in the filling and combustion tube is driven out by a stream of carbon dioxide before the sample undergoes combustion. Micro-bubbles still ascend the solution in the azotometer after passing carbon dioxide for some time. When the size of these is constant the combustion is started and is continued until micro-bubbles of the same size as those obtained previously are again obtained near the top of the liquid in the azotometer. The copper spiral is placed in the middle of the filling of the combustion tube in order to reduce the dissociation of issuing carbon dioxide into monoxide and oxygen. The volume of nitrogen in the azotometer is read at known temperature and pressure and from that volume is deducted 2% of the volume read, to allow for the volume of potash solution adhering to the walls of the azotometer.

The method, with occasional modification, is applicable to all nitrogenous organic substances. The amount of substance required for the analysis is 2 to 5 mg. and the volume of nitrogen obtained is 0.3 to 0.5 ml. The time required for the determination is about 20 minutes.

Volumetric Determination of Nitrogen by Micro-Kjeldahl Method. The principles of this method are:—(1) Destruction of the substance by heating with red phosphorus and hydriodic acid and then with sulphuric acid, mercuric acetate and potassium sulphate; the nitrogen is thus obtained as ammonium sulphate. (2) Steam distillation of the ammonia liberated by alkali. (3) Volumetric determination of the ammonia with $\text{N}/100$ acid. Very small amounts of ammonia are best determined by iodimetric determination of the excess of $\text{N}/100$ hydrochloric acid (using potassium iodide, potassium iodate and $\text{N}/200$ sodium thiosulphate) or colorimetrically with Nessler's reagent. The use of hydriodic acid in the decomposition renders the process applicable to hydrazines, dinitrohydrazones, osazones, oximes, nitro-, nitroso-, and even some diazo-compounds. The micro-Kjeldahl is not so generally applicable as the micro-Dumas method and fails completely, for example, with diazo-ketones of the general formula $\text{R} - \text{CO} - \text{CHN}$, since these split off almost all the elementary nitrogen with hydriodic acid in the cold. With diazo-compounds which easily split off nitrogen as an element, the Kjeldahl method may be used by first coupling the diazo-compound with phenol to form a stable azo-compound. For this coupling the substance is dissolved in three or four times its weight of phenol, heating on the water-bath. After cooling the Kjeldahl method is carried out in the usual way.

The advantages of the Kjeldahl method are found (1) in multiple determinations; (2) in determinations of the nitrogen in aqueous solutions (blood, urea, etc.); (3) in the analysis of heterocyclic compounds which in the micro-Dumas method tend to form nitrogenous charcoals burnt only with difficulty. A suitable steam distillation apparatus, described by I. K. Parnas and R. Wagner (*Biochem. Z.*, 1921, 125, 253), is recommended by Pregl and others.

Determination of the Halogens. The micro-Carius method is still used for the determination of bromine and chlorine by heating under pressure with nitric acid and silver nitrate, but a more rapid method and one more generally used is that due to Pregl. In Pregl's method the substance is ignited in a slow stream of oxygen. The gases produced pass over red hot platinum contacts and are finally absorbed in sodium carbonate solution containing sodium bisulphite, which reduces any halogenate or hypohalogenite to halide. The carbonate-bisulphite solution is spread over glass beads at the end of the combustion tube. At the end of the combustion the beads and tube are well

washed and the halide in this solution then precipitated as silver halide which is collected and weighed. The method is very accurate and the actual combustion takes about 35 minutes.

Chlorine and bromine may be determined alkalimetrically by the method of M. K. Zacherl and H. G. Krainick (*Mikrochemie*, 1932, *11*, 61). The organic material is oxidised, in a current of oxygen, with concentrated sulphuric acid in the presence of potassium dichromate and silver dichromate. Chlorine and bromine are thereby volatilised whilst iodine is quantitatively retained as iodate. The halogens are carried over by the oxygen into an absorption apparatus where they react with a measured amount of sodium hydroxide and concentrated hydrogen peroxide of 100 vol. strength. The unused alkali is titrated back using methyl red as indicator and a blank test on the acidity of the hydrogen peroxide is made.

Chlorine and bromine in the presence of each other may be determined by weighing all the halogen as silver halide, as in Pregl's method, then heating this residue with excess of ammonium iodide or bromide. The method depends on the thermal dissociation of the individual ammonium halides. If a mixture of silver chloride and silver bromide is heated with excess of ammonium iodide or bromide at 300° the total silver halide is transformed into silver iodide or bromide. From the weight of the silver chloride and bromide precipitate (first weighing) and the silver iodide or bromide formed (second weighing) the chlorine and bromine content can be calculated. The indirect determination of chlorine and bromine when both are present can be carried out accurately to $\pm 0.5\%$.

Iodine liberated in Pregl's tube combustion method is collected in dilute sodium hydroxide solution and oxidised with bromine to iodic acid. Excess of bromine is removed by steam or formic acid. Potassium iodide is added to the iodic acid formed and the liberated iodide is titrated with $N/50$ sodium thio-sulphate.

Determination of Sulphur. Sulphur in organic compounds may be determined gravimetrically or volumetrically. The gravimetric determination may be carried out thus:—

- (1) By Pregl's method of combustion in a stream of oxygen in the bead tube, as with halogens. The combustion takes about an hour. Here the products are absorbed in a solution of hydrogen peroxide which oxidises any sulphurous acid to sulphuric acid, the latter being precipitated as barium sulphate. The precipitate is collected in a micro-crucible and weighed. The crucible is fitted with a lid, and a capsule fits over the bottom; it is 14 mm. high and has a diameter of 12 mm. above and 11 mm. below. The filtering layer consists of iridio-platinum sponge, which completely retains barium sulphate precipitated in the cold at a comparatively high rate of filtration.
- (2) By Pregl's modification of the Carius method. The decomposition is carried out in the pressure tube with concentrated nitric acid and a crystal of barium chloride, the barium sulphate precipitated being weighed.

The essentials of the volumetric methods are as follows:—

- (1) The sulphuric acid formed in the bead tube may be titrated directly by Pregl's method, with $N/100i$ sodium hydroxide, if nitrogen and halogens are absent.
- (2) If nitrogen and halogens are present, nitric, hydrochloric and hydrobromic acids are formed in the combustion as well as sulphuric acid. The total acid is neutralised with sodium hydroxide in a quartz flask using phenolphthalein as indicator. After the addition of sulphuric acid in an amount exactly equivalent to that of the sodium hydroxide added, the volatile acids liberated are driven off by repeated evaporation on the water-bath with the formation of sodium sulphate, whilst the sulphuric acid originally present forms sodium bisulphate. Titration then of the bisulphate corresponds with that of the original sulphuric acid.

Determination of Phosphorus. The organic substance is destroyed either by fusion with sodium carbonate and potassium nitrate or by boiling with a mixture of nitric and sulphuric acids by which the phosphorus is oxidised to phosphoric acid. The acid is quantitatively precipitated with the ammonium molybdate reagent of N. v. Lorenz (*Z. anal. Chem.*, 1912, *51*, 161) and weighed as ammonium phosphomolybdate or determined volumetrically by dissolving the precipitate in excess of standard sodium hydroxide solution, boiling off the ammonia, and titrating back the excess of alkali. An analysis accurate to

$\pm 0.1\%$ is easily obtained, because the precipitate weighed is 63 times as heavy as the phosphorus which it contains. By the titration method one atom of phosphorus requires 28 equivalents of alkali.

If the organic substance also contains arsenic, then a mixture of phosphoric and arsenic acids is obtained by the decomposition. The latter must be removed before precipitation by reducing the pentavalent arsenic with hydrazine in hydrochloric acid and distilling off the trichloride in a slow stream of hydrogen chloride (R. Kuhn, *Z. physiol. Chem.*, 1923, 129, 64).

Determination of Arsenic and Antimony. Arsenic is determined as magnesium ammonium arsenate. Antimony is determined as the tersulphide. The two methods can be combined to separate and determine arsenic and antimony.—F. Hecht and M. v. Mack, *Mikrochimica Acta*, 1937, 2, 218.

Micro-titration of Calcium. Take a solution of the sample; ammonium oxalate and 1 drop of bromo-cresol purple are added. The pH is adjusted until the solution is of a grey colour, and after standing 1 hour, the liquid is centrifuged. The supernatant liquid is decanted and the precipitate suspended in ammonium oxalate and again centrifuged. The precipitate is dried and heated in a muffle-furnace at 475° to 525° for 20 to 30 minutes. The tube is placed in boiling water and hot boric acid solution added. The precipitate of calcium carbonate dissolves. The solution is diluted with water and titrated with sulphuric acid to the pH of a solution of boric acid of equal concentration, using Patterson's Indicator, with an external control.—A. E. Sobel and S. Sklarsky, *J. biol. Chem.*, 1938, 122, 665.

Determination of Alcohol in preparations. The solution suitably diluted to contain about 1 ml. of ethyl alcohol is mixed with 1.5 ml. of conc. sulphuric acid and titrated against potassium dichromate solution at 100° , using the leuco-base of methylene blue as external indicator.—A. I. Matin *et al.*, per *Brit. chem. Abstr. (B)*, 1940, 169.

General Groups. Microchemical methods are also used for the determination of special groupings in organic substances, including carboxyl, hydroxyl, amino, methoxyl or ethoxyl, *N*-methyl or ethyl, acetyl and benzoyl groups.

Physical Constants. Micro-methods have been developed and are much used for the determination of the common physical constants. An accurate boiling-point determination can be made with a fraction of a drop of liquid. This is contained in a capillary tube about 1 mm. bore which is drawn out very fine at the lower end and sealed in such a way that a minute bubble of air is enclosed below the liquid. This capillary is then attached with a drop of liquid on a glass plate fixed by a rubber band to a thermometer and immersed in a heating bath. On heating up, the air bubble expands slowly until the boiling-point is reached and then suddenly increases in volume, driving the drop of liquid to the level of the surface of the bath—the temperature at which this occurs is the boiling-point of the liquid tested. Micro melting-points are determined with a tiny fragment of material on a cover-glass heated on an electrically heated copper block which has a central hole so that the melting can be observed under a microscope. The method is particularly useful if the changes are observed with crossed Nicols. At the melting-point a sudden darkening occurs.

For description of apparatus and methods suitable for inorganic quantitative micro-analysis see Lectures by Professor H. V. A. Briscoe and Dr. J. W. Matthews, given March 1934 to Institute of Chemistry. *Other References:*—*Lehrbuch der Mikrochemie*, 2nd Edition, by Friedrich Emich; *Die Praxis der Quantitativen organischen Mikro-analyse*, by A. Friedrich; *Handbook of Chemical Microscopy*, by Chamot and Mason; *Recent Advances in Analytical Chemistry*, Vol. II (Micro-chemistry), edited by Dr. Ainsworth Mitchell.

SPECTROPHOTOMETRIC ANALYSIS

When light passes through any homogeneous transparent medium it emerges diminished in amount. Part of the light may be scattered at the surface, part scattered in the interior and part regularly reflected at the surfaces. The remainder of the light which is lost is said to be absorbed. It may be transformed into heat or

into fluorescent or phosphorescent light of different wavelengths from its own or it may cause ionisation or chemical action. The study of the absorption of light by substances in solution has proved of considerable assistance in analysis. Absorption spectra have been studied at all wavelengths of light—in the infra-red region, in the visible region and in the ultra-violet region. Largely due to the expense of the apparatus necessary, infra-red absorption spectra have not yet been so widely applied to technical problems as have absorption spectra in the visible and ultra-violet regions.

Quantitative absorption spectroscopy depends upon two important laws—Lambert's and Beer's.

Lambert's Law states that in passing through layers of equal thickness of a homogeneous material, equal fractions of the incident radiant energy are absorbed. Hence the proportion of the incident light absorbed by a particular material depends upon the thickness of the column of material in which absorption occurs. The diminution of the intensity of the incident light, $-dI$, occurring on passing through a small thickness, ds , of medium is proportional to the intensity of incident light and to ds . Hence $-dI \propto I \times ds$ or $-dI = k'I ds$ where k' is a constant depending on the medium and is termed the absorption coefficient. Rearranging $-\frac{dI}{I} = k' ds$ and integrating for a layer of thickness

where I_0 is the intensity when $s = 0$ and I is the intensity of the emergent beam, then

$$\log_e I_0/I = k's = \text{Extinction} = E$$

or in common logarithms $\log_{10} I_0/I = ks$ where $k = k'/2.303$.

Beer's Law states that the amount of light absorbed is proportional to the number of molecules in the light path. Thus a 10 cm. layer of $M/1000$ solution exerts the same effect as a 1 cm. layer of $M/100$ solution or a 1 mm. layer of $M/10$ solution. Beer's Law does not apply in all cases over wide variations in concentrations. The value of k therefore depends on the nature of the medium and on the concentration, so that $k = \epsilon c$ where ϵ is the molecular extinction coefficient when the concentration is expressed in gramme molecules per litre.

The extinction E is therefore given by $E = \log I_0/I = \epsilon cs$.

Both the optical density E and the molecular extinction coefficient ϵ are used in expressing the results of absorption measurements and since the absorption varies with the wavelength of light used (λ) results are usually set down graphically with ϵ or $\log \epsilon$ (or E or $\log E$) as ordinates against λ as abscissae. The logarithmic curves have advantage when ϵ and hence E varies over a wide range.

The value of the extinction E is therefore independent of the intensity of the light used, but is determined by the thickness, concentration and nature of the medium in the path of light. The expression E_s^c indicates the amount of light absorption occurring in passing through a layer of thickness s of a material in solution in concentration c .

Extinction Coefficient. $E_{1\text{ cm.}}^{1\%}$ is the commonly expressed form of the extinction coefficient and is readily calculable thus; if a 0.25 cm. layer of a 0.05 per cent. solution transmits 4/5 of the incident light at 325m μ , then

$$E_{0.25\text{ cm.}}^{0.05\%} = \log 5/4 = 0.0969$$

$$\text{and } E_{1\text{ cm.}}^{1\%} = \frac{0.0969 \times 1 \times 1}{0.25 \times 0.05} = 7.752.$$

The use of a standard method of expression of the extinction coefficient as $E_{1\text{ cm.}}^{1\%}$ renders easier the comparison of similar solutions and the uniform conversion into other expressions and is specially useful in dealing with mixtures or substances of unknown molecular weight.

The Spekker Photoelectric Absorptiometer. Measurement of absorption of light in the visible region of the spectrum is

involved in all colorimetric determinations but because of the inherent difficulties attendant on visual measurement the value of a photo-electric cell has become increasingly appreciated. Several instruments are available for photo-electric measurement of the absorption spectra at wavelengths in the visible region, and are sometimes referred to erroneously as photo-electric colorimeters. Since they do not measure colour but the absorption of light by the coloured mixture of solution and reagents they are in fact absorptiometers. One such instrument which is finding increasing application is the Spekker Photoelectric Absorptiometer. A 100-watt projector lamp, mounted in a cylindrical housing in the middle of the instrument and operated from the electric mains supply, is the source of light. A lens mounted in the housing to the right of the lamp, forms a parallel beam of light which passes through the specimen being examined and falls on another lens which forms an image of the lamp filament on the indicating photocell. A calibrated variable aperture mounted immediately in front of the lens system, enables the intensity of the light falling on the photocell to be varied by known amounts. Since there is an image of the filaments on the cell there is no change in the cell area illuminated when the aperture alters. The scale associated with the aperture is so calibrated that if R is the reading corresponding to a degree of opening such that the amount of light transmitted is $1/a$ of that admitted when the aperture is fully open then $R = \log a$, this function being chosen because it is approximately linear with the concentration of a solution, over small ranges. Light from the lamp also falls on a compensating photocell to the left of the instrument through a glass cell containing water, a filter, and an iris diaphragm by means of which the amount of light falling on the photocell can be varied. The two photocells are connected in opposition across a galvanometer so that when the photo-electric currents given by the cells are equal, the galvanometer shows zero deflection. If the light incident to the specimen is unfiltered the response of the indicating photocell with respect to selective absorption may be small. If, however, the light passing through the specimen is from that part of the spectrum which is being absorbed in greatest amount the response of the photocell will be considerably increased, a condition achieved by the use of filters which are available.

Technique. For comparison of the amount of light absorbed by two solutions the cell containing the more deeply coloured liquid is put into the beam and the calibrated variable aperture is opened to its fullest extent by setting the drum at zero. The iris diaphragm in front of the compensating cell is adjusted until the galvanometer shows zero deflection. The cell containing the less deeply coloured solution is then pushed along by means of a slide until it comes into the beam falling on the indicating photocell. The calibrated variable aperture is now adjusted by means of the drum until the galvanometer returns to zero and the reading of the scale associated with the calibrated variable aperture is

observed. Calibration curves are then drawn for drum readings against known concentrations of the material being examined. Provided concentrations and filters are chosen to give the optimum extinction, readings can be obtained corresponding with an accuracy in concentration of 1%.

Applications. Among practical applications of the photoelectric absorptiometer are:—

The precise determination of pH values by means of indicators for which an accuracy of 0.02 to 0.04 pH has been achieved.—Lothian, *Trans. Far. Soc.*, 1937, 33, 1239.

The determination of small quantities of manganese, chromium, molybdenum, vanadium, phosphorus, iron, titanium, copper.—E. J. Vaughan, *The Use of the Spekker Photoelectric Absorptiometer in Metallurgical Analysis*, Institute of Chemistry Lecture, London, 1941.

The estimation of vitamin A and carotene with the photoelectric absorptiometer, utilising the antimony trichloride reaction.—C. J. Koehn and W. C. Sherman, *J. biol. Chem.*, 1940, 132, 527; also Dann and Evelyn, *Biochem. J.*, 1938, 32, 1008.

The determination of phosphorus by measurement of the spectral transmission of the blue colour obtained by reduction of the sodium molybdate and phosphorus complex with stannous chloride, in a photoelectric absorptiometer.—T. D. Fontaine, *Industr. Engng Chem. (anal. Edn.)*, 1942, 11, 77.

The determination of iron by measurement of the spectral transmission in a photoelectric absorptiometer of the α : α' -dipyridyl compound.—R. A. Koenig and C. R. Johnson, *J. biol. Chem.*, 1942, 143, 159.

The determination of 17-ketosteroids in urine extracts by measurement of the absorption by solutions treated with alkaline *m*-dinitrobenzene in alcohol, in a photoelectric absorptiometer.—A. F. Holtorff and F. C. Koch, *J. biol. Chem.*, 1940, 135, 377.

The determination of vitamins D₂ and D₃ by modification of the colour test of Brockmann and Chen (*Analyst*, 1936, 629) by use of a reagent consisting of antimony trichloride in chloroform with addition of acetyl chloride and measurement of the light absorption at 500m μ spectrophotometrically.—C. H. Nield, W. C. Russell and A. Zimmerli, *J. biol. Chem.*, 1940, 136, 73. The photoelectric absorptiometer is also known to be quite suitable with the appropriate filter for this measurement.

Aneurine may be determined by measurement of the absorption in the reddish solution of variable tint in a photoelectric absorptiometer, produced by the Peters colour test, using an alkaline solution of diazotised sulphanilic acid containing formaldehyde.—A. L. Bacharach (Private communication).

Many metals may be determined by the use of organic reagents, the determination of small quantities being possible by measurement of the light absorption in a photoelectric absorptiometer and preparation of calibration curves.

Ultra-violet Absorption Spectroscopy. Many substances in solution, both inorganic and organic, exhibit pronounced selective absorption at wavelengths in the ultra-violet region, 2000 to 4000 Å being the range commonly employed. Ultra-violet absorption spectroscopy has, therefore, found wide application in the solution of chemical problems, and the refinement of photometers for use with spectrographs has brought about the required accuracy in quantitative work. Many instruments are available for absorption spectroscopy in the ultra-violet region, including those for visual use where measurements are made by the aid of photography, and those employing photoelectric methods of measurement of light intensity. Widely differing though they are in detail of design, all spectrographs for use in the ultra-violet region have certain features in common—light from a suitable source passes through the solution being examined, then through

a collimating quartz lens, then through a quartz biprism, so constructed as to eliminate the double refraction of quartz, and finally through a lens system on to a photographic plate. By employing a constant deviation prism, the collimator and the telescope can be rigidly fixed, and rays of successive wavelengths brought into view by rotation of the prism on a vertical axis by means of a drum graduated directly in wavelength. In order to overcome the effects of variation of light intensity at different wavelengths a suitable device is mounted in front of the spectrograph. Such an apparatus is the Spekker spectrophotometer.

The Spekker Spectrophotometer. Light from a suitable source of ultra-violet rays passes towards the inner edges of two quartz rhombs from which the beams are diverted upwards and downwards respectively, to be reflected forward through two quartz tubes, in one of which is placed the absorbing liquid and in the other the liquid with which comparison is to be made. For example, in the one tube there may be a substance in solution, and in the other tube the solvent only. The beams from the rhombs pass, the one through a fixed rectangular aperture and the other through an aperture the size of which can be varied by means of a micrometer screw, rotated by a drum, with an index which travels along a helix in the drum. After passing through the absorbing substance and the non-absorbing substances the beams pass through quartz lenses, the focal length of which is such that an image of the light source is formed on the face of the slit of the spectrograph. A second pair of quartz rhombs is arranged to bring the beams of light together on to the slit, in such a way, that the image falling from the top rhomb and that from the bottom rhomb on the slit form a complete image of the light source. The spectrophotometer is rigidly attached to the spectrograph. Several photographs are then taken, one below the other by raising the dark slide by means of a handle, with various widths of the adjustable aperture. In this manner a number of pairs of spectra are obtained and since spectrographs can be obtained adjusted for working with ranges of wavelengths, a wavelength scale can be printed in the spectrum and is developed on the plate with the spectra. One spectrum of each pair is cut down in intensity uniformly along its length by a known amount, whilst the other has its intensity locally reduced by reason of the absorption of the liquid being examined. There are certain places in the two spectra where the intensities match, these are noted and the wavelength corresponding to them observed. Knowing the width of the aperture corresponding to that particular pair of spectra the absorption of the liquid can be deduced. Each position of the aperture corresponds to a definite reduction in intensity of light, and so the drum can be graduated directly in extinction coefficients. Thus can be constructed a graph of the variation of extinction coefficient with wavelength, inflections in the graph, especially maxima, corresponding with characteristics of the substance under examination. Various sources of ultra-violet

light are used, commonly employed being a condensed spark between nickel or steel alloy electrodes. For finer details a continuous spectrum is desirable, and may be obtained by a hydrogen discharge lamp or a high tension under-water spark between tungsten electrodes.

For the principles governing the choice of photographic plates, details concerning the purification of solvents and construction of cells see *The Practice of Absorption Spectrophotometry*, Twyman and Allsopp, London, 1934. Hexane, heptane, and cyclohexane are commonly used solvents for ultra-violet spectroscopy, suitably purified materials for such work being commercially available.

Applications. Ultra-violet spectroscopy has been of value in elucidation of structural problems in inorganic as well as organic chemistry. A recent survey of applications to inorganic chemical problems is included in *Absorption Spectra*, R. A. Morton, *Ann. Rep. Chem. Soc., Lond.*, 1941, pp. 9-14. Wider applications to technical problems have resulted from the study of ultra-violet absorption spectra of organic substances. Since extinction coefficients of organic compounds generally can vary over a wide range it is important, especially with the more transparent substances, to have the material as pure as possible though many applications to mixtures have been successful. All saturated hydrocarbons, cyclic or acyclic, show negligible absorption. If a group T is introduced into one saturated hydrocarbon and the new molecule is still transparent, the group is itself transparent and will not endow any other hydrocarbon with selective absorption. If, on the other hand, a group A confers selective absorption on one saturated hydrocarbon it will do so to all saturated hydrocarbons and is itself an absorbing group or chromophore. Among the more important chromophores are $>C=O$, $-CH=O$, $>C=C<$, $-CH=N-$, $-N=N-$. In many homologous series, e.g., $CH_3(CH_2)_nA(CH_2)_nCH_3$ the absorption spectrum is independent of n and if a compound contains two similar chromophores separated by CH_2 there is little change in absorption. Among pairs of substances indistinguishable spectroscopically are: succinic and glutaric acids; diphenoxymethane and diphenoxydecane; ethylbenzene and benzylethylene. Compounds containing a single ethylenic linkage, e.g., $CH_3CH=C(CH_3)_2$, show strong absorption in the extreme ultra-violet, the band being displaced to longer wavelengths with the introduction of conjugation. With increasing conjugation the absorption is displaced again in the direction of longer wavelengths and increases in intensity.

Technical Applications. Among technical applications of ultra-violet spectroscopy are examination of chemical warfare materials, colloids and metallic films, dyestuffs and textiles, electrical industry materials, foodstuffs and fermentation products, glasses and glazes, hydrocarbons, fuels and mineral oils, iron and steel, leather, oils and fats, paints and pigments, paper and cellulose, printing inks, resins and plastics, rubber, sugars and starches, biological materials, vitamins and hormones, terpenes and essential oils, purines and alkaloids, proteins and enzymes. A bibliography and brief abstracts of

866 papers published between 1932 and 1938 are given in *Absorption Spectrophotometry and its Applications*, O. J. Walker, London, 1939.

Pharmaceutical and Analytical Applications. Among applications of ultra-violet absorption spectroscopy of pharmaceutical and analytical interest and importance are the following:—

Vitamin A. Vitamin A exhibits selective absorption of ultra-violet light a maximum absorption occurring at $325\text{ m}\mu$ which forms the basis of a method for its determination. The method is recognised by the British Pharmacopœia 1932 (First Addendum 1936, and Second Addendum 1940, 21). From the value of $E_{1\text{ cm}}^{1\%}$, the value of vitamin A activity in international units per gramme is obtained by multiplying the ultra-violet absorption by a factor declared by the Permanent Commission on Biological Standardisation of the Health Organisation of the League of Nations as the factor to be used. The factor at present in use is 1600, but in the United States of America higher factors are used. Although the factor for converting $E_{1\text{ cm}}^{1\%}$ at $325\text{ m}\mu$ into international units per gramme may be controversial, J. R. Edisbury (*Analyst*, 1940, 492) suggests that the higher factors used in America originated through inefficient utilisation of the carotene when the U.S.P. Reference Oil was calibrated. Edisbury reports that the conversion factor of 1600 is applicable for fish liver and visceral products and of 1200 for whale liver products. Purified crystalline vitamin A examined by H. N. Holmes and R. E. Corbet (*J. Amer. chem. Soc.*, 1937, 59, 2042) had $E_{1\text{ cm}}^{1\%}$ 2000. Vitamin A in oils is usually determined by measurement of the absorption at $325\text{ m}\mu$ of a solution of the unsaponifiable fraction of the oil in cyclohexane. The effect of the solvent used on the value of $E_{1\text{ cm}}^{1\%}$ has been discussed by D. C. M. Anderson and N. Evers (*Analyst*, 1941, 106) who consider cyclohexane to be preferable to isopropyl alcohol or absolute alcohol. Although in many cases vitamin A may be determined by measurement of the ultra-violet absorption on a dilution of the oil itself, yet very misleading results may occur due to irrelevant absorption caused by glycerides or coloured materials in the oils. It is therefore desirable to determine vitamin A on the unsaponifiable fraction of the oil, a suitable method being that based on the Society of Public Analysts recommendation (*Analyst*, 1933, 203) as adapted by J. R. Edisbury (*Analyst*, 1940, 492). For artificially vitaminised oils, provided samples of the oil before vitaminisation are available for use as control, reliable results can be obtained by direct measurement of $E_{1\text{ cm}}^{1\%}$ at $325\text{ m}\mu$ on dilutions of the vitaminised oil and control in cyclohexane.

(For further information see *Absorption Spectra of Vitamins, Hormones and Co-Enzymes*, by R. A. Morton, 2nd Edn., 1942.

For the spectrophotometric determination of vitamin A a simplified apparatus—the VITAMETER—is available, depending on the use of a screen of fluorescent material.

Vitamin B₁. Aneurine hydrochloride may be determined in solutions by measurement of the absorption at $247\text{ m}\mu$. The accuracy of this spectrophotometric method is discussed by K. H. Coward (*Quart. J. Pharm.*, 1941, 329).

Calciferol. The photolability of calciferol necessitates special precautions for determination of its absorption spectrum $E_{1\text{ cm}}^{1\%}$ at $265\text{ m}\mu$ being not less than 460 (Addendum 1936 to British Pharmacopœia 1932). S. K. Crews and E. L. Smith (*Analyst*, 1939, 568) recommend a continuous flow method as the most accurate means of determination.

Oils. Halibut-liver oil may be distinguished from mixtures containing whale-liver oil by the fact that halibut-liver oil shows a distinct peak in the absorption curve at $328\text{ m}\mu$, the value at 295 to $300\text{ m}\mu$ being not more than 75 per cent. of the $328\text{ m}\mu$ values. Whale-liver oil does not show the peak at $328\text{ m}\mu$, the value at $300\text{ m}\mu$ being approximately the same as at $328\text{ m}\mu$, so that the curves show practically a straight line between these two points. Admixture of whale-liver oil with halibut-liver oil would cause the absorption peak at $328\text{ m}\mu$ to be displaced towards the lower wavelengths (Haines and Drummond, *Analyst*, 1935, 335). Results of the examination of 60 samples of halibut-liver oil are quoted by Elvidge (*Quart. J. Pharm.*, 1940, 223). The Second Addendum (1940) to the British Pharmacopœia 1932 includes as the test for purity of halibut-liver oil the requirement that the ultra-violet absorption

at 300 $m\mu$ is not greater than 75 per cent. of that at 328 $m\mu$ (absence of whale-liver oil).

Since the extinction coefficient of olive oil at 268 $m\mu$ is 11, while that of tea seed oil is 690, spectrophotometric examination is sometimes of value for the detection of tea seed oil in olive. The presence of 1% of tea seed oil produces a distinct inflection in the absorption curve of olive oil at 268 $m\mu$ and of 5%, a distinct maximum absorption. (Judd Lewis, *Analyst*, 1935, 60, 16; but see also Elvidge, *Quart. J. Pharm.*, 1941, 147.) For value of ultra-violet absorption spectra in assessing quality of linseed oils see J. H. Mitchell and H. R. Kraybill (*Industr. Engng Chem. (anal. Edn.)*, 1941, 13, 765).

Stilbæstrol and Hexæstrol. A maximum occurs in the absorption curve for stilbæstrol at 239 $m\mu$, $E_{1\text{ cm}}^{1\%}$ being 600. Hexæstrol exhibits maxima at 229 $m\mu$ and at 281 $m\mu$. These have been used for the determination of stilbæstrol and hexæstrol in tablets.—(Elvidge, *Quart. J. Pharm.*, 1939, 347.

Steroid Hormones and their Derivatives. Spectrophotometric methods have been applied to the determination of œstradiol benzoate and progesterone in oily solutions.—Elvidge, *Quart. J. Pharm.*, 1939, 347.

Alkaloids. Absorption spectra for many alkaloids have been published, including cocaine and its analogues, hyoscyamine, atropine and hyosine, ephedra alkaloids, morphine and its derivatives (Ellinger, *Tabule Biologica*, 1937, xii, 313; 1938, xvi, 290, 292, 306) and have been re-examined by Elvidge (*Quart. J. Pharm.*, 1940, 224) who has successfully applied them to the assay of powdered ephedra, powdered ipecacuanha and opium pill (*Quart. J. Pharm.*, 1941, 134). Among other substances of pharmaceutical interest and importance Elvidge has found that the absorption spectra of the following are likely to be of value; barbitone, phenobarbitone, diallylbarbituric acid, sodium methyl-ethylbutylbarbiturate, root-growth-stimulating naphthalene and indole derivatives, hexylresorcinol, menaphthone, aneurine hydrochloride and sulphonamides (*Quart. J. Pharm.*, 1940, 219; 1941, 134). R. E. Stuckey has also examined under various conditions the absorption spectra of barbituric acid, barbitone, phenobarbitone and their methyl derivatives, but points out that ultra-violet absorption spectra will not readily differentiate between barbitone, phenobarbitone or their 1-methyl derivatives, although the peak in alkaline solution will be of value in analysis for determining small amounts of these substances in viscera, urine and cerebrospinal fluid (*Quart. J. Pharm.*, 1940, 312; 1941, 217).

As a result of the examination of an increasing number of samples of pharmaceutical substances, the application of spectrophotometric analysis to their determination in mixtures and other preparations which are otherwise difficult to examine, is likely to become of increasing importance.

Other References:—R. A. Morton, *Practical Aspects of Absorption Spectrophotometry*, Two Lectures to Institute of Chemistry, London, 1938; T. R. Hohness and V. R. Potter, *Spectrometric Studies in Relation to Biology*, *Ann. Rev. Biochem.*, 1941, 10, 509; W. R. Brode, *Chemical Spectroscopy*, London and New York, 1939; *Spectrophotometric Terms and Symbols*, *Analyst*, 1942, 164, 227.

CHEMICAL TESTS & MICROSCOPIC METHODS FOR THE EXAMINATION OF URINE, BLOOD, FÆCES, STOMACH CONTENTS, &c.

URINE

The constituents of urine are derived in part from material absorbed from the digestive tract but not required by the body, and in part from the end products of metabolic processes conveyed in the blood stream from the various tissues to the kidneys. In practically all the more serious pathological conditions marked changes occur in the nature or the proportion of the constituents of urine and the examination is therefore of great diagnostic value in ascertaining the presence or the progress of disease.

The yellow colour is due to pigments, e.g., urochrome. Blood pigment gives a red or brown, smoky colour. Urine which changes to a red colour on adding alkali is sometimes passed by patients taking phenolphthalein. Dark black urine suggests alkaptonuria, melanotic tumours or phenol poisoning. Bile pigments give an orange or brown colour, the colour being imparted to the froth on shaking. A port-wine colour is given in the presence of an excess of porphyrins. An appreciable amount of methæmoglobin imparts a brown colour. A blue or green colour is usually due to the taking of methylene blue. Very rarely it is due to the presence of indigo derived from an excess of indican.

The normal reaction is slightly acid (pH 6.0), due partly to acid phosphates and partly to free organic acids. Urine may become slightly alkaline after a full meal. On standing, it becomes alkaline owing to decomposition of urea and formation of ammonia. A similar marked alkalinity of freshly voided urine may be due to ammoniacal decomposition and may occur in severe cases of chronic cystitis.

The specific gravity of urine at $60^{\circ}F$. is usually between 1.015 and 1.025. It is low in chronic interstitial nephritis, diabetes insipidus and many functional nervous disorders. It is high in fevers and diabetes mellitus.

The total solids of the urine of a healthy adult amount to about 60 grammes or 950 grains per diem. A quick clinical method of determining the total solids is to multiply the last two figures of the specific gravity by the number of ounces voided and to add one-tenth of the product. This gives the amount in grains, e.g., $45 \times 20 = 900 + 90 = 990$ grains.

For quantitative examinations, a sample of the 24 hours excretion should be examined. For most qualitative examinations the first morning specimen is the best. In the case of female patients, where a microscopical examination is required, a catheter specimen should be obtained if possible.

Microscopic Examination.

The chief objects to be sought for are blood, pus, epithelium, casts, chemical deposits (crystalline or amorphous) and parasites. The centrifuged deposit or sediment should be used; a drop on a slide is covered with a cover slip and studied with a 2/3 and 1/6 inch objective. For accurate estimation of red blood cells or pus, the cells in the fresh uncentrifuged urine should be counted in a Fuchs Rosenthal cerebrospinal fluid counting-chamber. (*Blood is dealt with on p. 645.*)

Epithelium.

Epithelial cells are present in normal urine and have no pathological significance. They are larger than pus cells, rarely round, usually non-granular and have a small central nucleus. It is impossible to determine the region of the urinary tract from which epithelial cells have been derived, but a rough classification may be made. Angular squamous epithelium comes from the vagina. Cells from the ureter or pelvis of the kidney are small and round, resembling polymorphonuclear leucocytes except that their nuclei are definitely round. Such cells are commonly seen in specimens collected by ureteric catheterisation and must be distinguished from pus corpuscles. Fragments of tumours of the urinary tract should be teased out and stained, or, better, cut into microscopic sections. The recognition of isolated cells from a neoplasm is impossible.

Chemical Deposits.

The detection of a crystalline or amorphous deposit in the urine is no evidence that this substance is being excreted in excess, and in a case of calculus the chemical composition of the urinary sediment does not necessarily indicate the nature of the stone.

In acid urine, the only urinary deposits likely to be found are urates, uric acid, calcium oxalate, stellar phosphates and cystine. Urates are amorphous, brown or pink, and disappear on warming. Uric acid appears in many different forms as yellow or brown crystals which are soluble in sodium hydroxide (or lithium carbonate) and reprecipitated by hydrochloric acid. Calcium oxalate occurs as clear, colourless, octahedral crystals with an envelope appearance, or as dumb-bell shaped crystals. The crystals are insoluble in acetic acid but readily soluble in hydrochloric acid. Stellar phosphates are composed of calcium hydrogen phosphate and are readily soluble in acids. They appear when the urine is nearly neutral. They form long, narrow, flat prisms which are frequently collected in bunches or rosettes, or which may be fine and feathery. Cystine occurs only in patients with the rare congenital abnormality of cystinuria. The crystals occur as irregular hexagons with clear-cut straight sides; they are insoluble in acetic acid and ether, but soluble in hydrochloric acid and in ammonia and other alkalis. They occur only in acid urine.

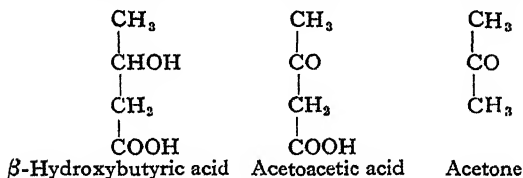
In alkaline urine, the commonest deposits are ammonium urate, amorphous phosphates, triple phosphates, calcium carbonate and calcium oxalate. Stellar phosphates sometimes occur. Ammonium urate forms a brownish deposit, soluble on warming. Amorphous phosphates form a white amorphous deposit, soluble in acetic acid. Triple phosphates form clear colourless crystals of various shapes and sizes, the typical ones being shaped like a knife-rest; they are soluble in acetic acid. Calcium carbonate forms a white deposit which may be amorphous or consist of dumb-bell shaped crystals. It dissolves in acid with effervescence.

In cases of acute liver atrophy, leucine and tyrosine may be found in the urine. Leucine crystals appear as yellow spheroids, with radial and concentric striations, and are soluble in acids and alkalis. Tyrosine forms brush-like tufts of fine needles which are soluble in ammonia and hydrochloric acid, and insoluble in acetic acid.

The following constituents of urine are dealt with in approximately alphabetical order.

Acetone and Allied Bodies

The bodies which often form abnormal urinary constituents comprise acetone, acetoacetic acid and β -hydroxybutyric acid. Their presence is usually due to the incomplete metabolism of fat, as for example when the tissues are deprived of carbohydrate. The chemical relationship between them is shown thus:—



Quite probably β -hydroxybutyric acid and acetoacetic acid are normally formed in the body during the splitting up of fat, but they are then fully oxidised.

It may be observed that the clinical significance of either acetoacetic acid or acetone (β -hydroxybutyric acid is not usually tested for) is the same.

Ketosis. This term is applied to the presence of acetone bodies in the blood or urine in abnormal quantities, their presence in the blood constituting ketonæmia and in the urine ketonuria. Ketosis is a symptom and not a disease and may arise from any condition which deprives the body of adequate carbohydrates in a combustible state. In the absence of carbohydrates fats are incompletely oxidised which results in the over-production of ketone bodies. Ketosis should not be confused with acidosis since its occurrence does not necessarily infer an acid condition either of the blood or urine. The most important disease in which ketosis is likely to appear is diabetes, in which the cause is primarily an inability to utilise the glucose resulting from carbohydrate ingestion, but in practice ketosis is more commonly met with in non-diabetic than in diabetic conditions. Starvation will produce ketonuria, even the temporary starvation due to prolonged vomiting, especially in children.

Qualitative Tests for Acetone Bodies.

Gerhardt's test consists in adding 10% ferric chloride solution drop by drop to the urine. At first a precipitate of ferric phosphate appears, and when this seems to have reached a maximum it should be filtered. If the filtrate shows no colour, a few more drops should be added, and the development of a red colour shows the presence of acetoacetic acid. The colour should be compared with that obtained on adding ferric chloride to normal urine. A positive reaction indicates a severe degree of ketosis but a negative reaction does not signify that the patient is free from the dangers of ketosis. This is partly owing to the relative insensibility of the reaction and partly owing to the fact that acetoacetic acid is converted on standing into acetone, which does not respond to Gerhardt's test. Various drugs cause a similar coloration with ferric chloride, but such fallacies are easily overcome by boiling another sample of the urine prior to testing. If the colour is due to acetoacetic acid, a negative reaction will be given by the boiled urine because the acid is volatile. The colour due to drug derivatives on the other hand is not affected by previous boiling of the urine.

Rothera's test for acetone and acetoacetic acid is a much more delicate test for ketosis. About 20 ml. of urine should be saturated with ammonium sulphate and a few drops of a freshly prepared dilute solution of sodium nitroprusside, $\text{Na}_2\text{Fe}_2(\text{CN})_{10}(\text{NO})_2 \cdot 4\text{H}_2\text{O}$, added (soluble 1 in $2\frac{1}{2}$). 2 or 3 drops of 10% ammonia are added and the tube shaken. If the reaction is positive, a delicate permanganate tinge develops and gradually deepens. A brown colour does not constitute a positive reaction.

The amount of acetoacetic acid can be judged by the depth of the colour and the rapidity with which it develops. A quick strong reaction corresponds to 0.25% and a slow weak reaction to 0.0005%. Intermediate reactions indicate proportional concentrations of acetoacetic acid (Kennaway). There are no fallacies in Rothera's test. A moderately strong positive Rothera's test is not of such grave importance as a positive Gerhardt's reaction.

Legal's test is similar. The nitroprusside is added to the specimen or to a distillate made slightly alkaline with potash. A red colour, changing rapidly to yellow, is positive. Acetic acid added gives a reddish-violet colour changing to blue.

The limiting concentration in Legal's test is, for acetoacetic acid, 6 mg. per litre, and for acetone 100 mg. per litre.—*J. chem. Soc. Abstr.*, 1925, 1490.

Iodoform Test. Distil the sample, make the distillate alkaline with potash, and add a little iodine solution (not an alcoholic solution). The formation of iodoform, recognised by the yellow turbidity and the odour, indicates presence of acetone. *Microscopic examination* is more conclusive than the odour.

Determination of acetone in urine—formation of iodoform in the usual way converting into silver iodide, and weighing.—*Yearb. Pharm.* 1919 56.

Scott-Wilson Test. Place one drop of Scott-Wilson reagent (see p. 57) on a microscopic slide and invert slide to form a hanging drop. Place the slide over the mouth of a flask containing the urine or blood and leave for 2 minutes. When acetone is present, the drop shows a fine white clouding or precipitate. A rough estimation of the amount of acetone present may be obtained as follows:—

| | | |
|-----------------------|-----------------------|--------|
| Faint trace | Opalescence | 0.005% |
| Trace | Faint turbidity | 0.01% |
| Moderate trace | Turbid | 0.025% |
| Heavy trace | More marked | 0.05% |
| Moderate amount | Very marked | 0.075% |
| Large amount | Precipitate | 0.1% |

Compare by setting up a series of tubes containing acetone from 0.1% down to 0.005%.—A. Wallhauser, *J. Amer. med. Ass.*, ii/1928, 21.

Riegler's Test. Differentiates acetone and acetoacetic acid in urine. 10 ml. of the specimen is acidified with 5 drops of 30% acetic acid and 5 drops of Lugol's iodine solution is added. Shake out with 2 to 3 ml. of chloroform. No colour appears if acetoacetic acid is present. The iodine is absorbed by the acetoacetic acid, forming a colourless compound.

Hurtley's Test for Acetoacetic Acid. To 10 ml. of urine add 2.5 ml. of concentrated hydrochloric acid and 1 ml. of 1% sodium nitrite. Shake and allow to stand two minutes. Add 15 ml. of strong ammonia followed by 5 ml. of 10% ferrous sulphate or a solution of ferrous chloride of equivalent strength (2 g. Fe in 100 ml.). Shake and pour into a 50 ml. Nessler glass. Do not filter. Violet colour forms slowly.

Acetone does not respond to the test, which is exceedingly delicate. It is assumed that isonitroacetone is first formed which then colours with the ferrous sulphate. The test can be rendered quantitative colorimetrically.

Iodine Absorption Test, Bela and Ondrovich. 5 drops of acetic acid 50% is added to 5 ml. of urine, then 1 drop of 1 in 500 methylene blue or *q.s.* to give blue tint. Titrate with N/10 iodine solution until a red tint appears— $2\text{I} = \text{CH}_3\text{CO} \cdot \text{CH}_2\text{COOH}$.

Iodic Acid Test. Add to 1 or 2 ml. of normal urine, 2 ml. of 10% iodic acid solution and 3 ml. of chloroform. Uric acid, etc., reduces the iodic acid—the chloroform becoming coloured with the iodine. Add 10 ml. of the specimen to be tested, and shake thoroughly. If acetoacetic acid is present the colour disappears, if absent it is intensified.

β -Hydroxybutyric acid may be extracted from the specimen with ether, and gives a reddish-violet colour with ferric chloride—the acetoacetic acid gives approximate index of the content of this acid. It occurs only if acetoacetic acid is also present. The specimen may be fermented to remove sugar, precipitated with lead acetate and ammonia; if the filtrate is levorotatory, β -hydroxybutyric acid is probably present.

Quantitative Estimation of Acetone. Normally from 20 to 50 mg. of total acetone bodies (calculated as acetone) are excreted in the urine daily. Of this from 3 to 15 mg. are in the form of acetone and acetoacetic acid and from 20 to 30 mg. as β -hydroxybutyric acid. In disease conditions the ketonuria is

not of much significance until more than 2 g. of total acetone bodies are excreted in the 24 hours.

A 50 ml. Erlenmeyer flask is provided with a tightly fitting cork from the under surface of which is suspended by a pin a pencil of compressed cotton wool as used by dentists. The urine is made acid to congo red by adding sulphuric acid solution (1 : 1) and 0.5 ml. is pipetted on to the cotton wool pencil. 0.5 ml. of 5% sodium bisulphite solution is placed in the flask, and the urine sample is suspended about 1 cm. above its surface. The flask is heated for 15 minutes in a boiling water-bath. The cork and cotton roll are removed, 1 ml. of water and 1 ml. of Nessler's reagent added, and the turbidity produced is compared in test-tubes with the turbidity produced by treating known amounts of acetone with 1 ml. of water, 0.5 ml. of 5% sodium bisulphite solution and 1 ml. of Nessler's reagent. The full turbidity is only developed after 15 minutes.—J. C. Abels, *J. biol. Chem.*, 1937, 119, 663.

Mix 1 ml. of hydrochloric acid and 10 ml. of urine. Place in a Pyrex test tube and stopper. Immerse in boiling water for 3 minutes and then in cold water. Place 5 ml. of alkaline mercuric reagent (prepared by mixing equal parts of sodium hydroxide solution, sp. gr. 1.33, and a solution containing mercuric chloride 10.8% and potassium iodide 28.8% in water) in the receiver of a special apparatus designed by Fleury (*J. Pharm. Chim.*, 1934, 20, 319). Warm the distillation apparatus, connected with the two reservoirs each containing 3 g. of barium sulphate, at 37° to 38° for $\frac{1}{2}$ hour. Introduce 2 ml. of the prepared urine drop by drop into the barium sulphate and insert the stopper. After at least 45 minutes at 37° to 38° place the apparatus in cold water for 10 minutes. Add 3 ml. of hydrochloric acid and mix by rocking. After 5 to 6 minutes add 5 ml. of N/25 iodine solution and 1 ml. of sodium hydroxide solution. Mix, stand for 15 minutes in cold water, add 3 ml. of diluted hydrochloric acid (1 : 1) and 5 ml. of water. Immediately titrate the excess iodine with N/50 thiosulphate solution. Carry out a blank determination. The difference in the burette readings $\times 0.1934 \times 1.1$ gives the mg. of acetone in 2 ml. of urine.—P. Fleury and J. Carbou, *J. Pharm. Chim.*, 1938, 28, 102.

Albumin

Albuminuria may occur in healthy individuals, as functional or postural albuminuria. The albuminuria may disappear when the patient is at rest, to reappear on taking exercise or even on assuming the erect posture. In pathological conditions, albuminuria is nearly always accompanied by tube casts and under these circumstances it points to organic disease of the kidney, or to severe irritation or circulatory changes in the kidney.

(Renal function tests are dealt with on p. 661 *et seq.*)

The amount of albumin detected at any time does not measure the degree of the albuminuria. The degree of albuminuria bears no relation to the amount of renal damage.

The mere presence of albumin in the urine of adolescents need not be regarded so gravely as was once the case, provided there are no other signs of renal or constitutional disability. One in every twenty male adolescents exhibits the condition, which may persist throughout life without detriment to physical efficiency. The after-rest specimen is usually free from albumin. The condition is not associated with any particular type of youth or man.—H. H. Bashford, *Lancet*, ii/1926, 1305-7.

Pure albumin free from globulin can be isolated from the urine by repeated precipitation with sodium sulphate. In cases of albuminuria of pregnancy (and proteinuria not associated with pregnancy) the albumin had a specific rotation averaging -55.81° , whereas in eclampsia there were two groups averaging -56.37° and -38.5° . Accordingly, it is suggested that in certain types of eclampsia the urinary albumin may be mainly *lactalbumin*, and that eclampsia may be an anaphylactic reaction due to the circulation in the blood of this foreign protein, in the production of which the mammary gland may be an important factor.—A. Hynd, *Lancet*, ii/1925, 911, 925.

Tests for Albumin in Urine.

The simplest and most accurate test is to boil the top of a column of urine in a test-tube, and if a turbidity appears in the part boiled add a drop of 5 to 10% acetic acid. Phosphates are dissolved but albumin remains. The delicacy of the test depends on the contrast between the upper and lower region and the urine must be filtered if it is not clear before the test is conducted.

Salicylsulphonic Acid Test. This has the advantage that it can be performed in the cold. An equal volume of urine is placed in two test-tubes and 2 or 3 drops of 25% solution of salicylsulphonic acid added to one of them.

In the presence of albumin the liquid in the tube to which salicylsulphonic acid was added will appear turbid compared with the control.

The urine of patients who have been given iodoxyI gives a false positive reaction with salicylsulphonic acid but not with the boiling test.

It is an extremely precise, reliable, and quick test, giving a dense white precipitate.

In confirmation note the following:—

Albumin, globulin, myosin, etc., coagulate on heating.

Albumoses dissolve on heating, and reappear on cooling.

It is not affected by phosphates, bile, urates or alkaloids.

Pure peptone is not precipitated, only the intermediate products between albumin and peptone, in solutions saturated with ammonium sulphate.

Colorimetric Biuret Method for determination of proteins. The proteins are precipitated by trichloroacetic acid; the precipitate is dissolved in sodium hydroxide solution and copper sulphate solution is added. The resulting purple solution is centrifuged to remove copper hydroxide after which it is matched against standard biuret solutions or coloured glass standards prepared by matching in the Lovibond comparator a series of solutions of known protein content prepared from normal human serum and treated by the same biuret technique.

Picric Acid Solution (Esbach's Reagent). Picric acid 10 g., citric acid 20 g., dissolved in about 900 ml. of boiling water; cool and add water to 1000 ml. This reagent is used for the approximate determination of albumin by an albuminometer which is about six inches long and 0.6 inch in diameter; the graduations on it are the results of experiment and indicate approximately 0.1% up to 0.7% of albumin.

By comparison with a standard dried albumin solution, 1 in 1000, and by heating both to 180°F. and centrifuging, the process can be terminated in a few minutes.

Mann warns against the voluminous precipitate which one occasionally gets with Esbach's reagent, giving a fictitious estimation. Many albuminous urines

give a pale blue with the biuret reaction without any tendency to violet; others will give a reddish-purple. Such urine indicates by the reddish colour some hydrolytic change and will give the incorrect reading referred to.

For exact determinations, albumin should be precipitated by some suitable reagent, itself nitrogen-free, e.g., phenol or tannin, and the washed precipitate, dried and weighed, or better, the nitrogen contained in it should be estimated by a Kjeldahl analysis, the amount of nitrogen found being multiplied by the factor 6.3 to obtain the amount of proteins.

N.B.—Methylene blue precipitates picric acid solution in case of patients undergoing treatment with this compound.

The administration of alkaloids may cause urine to give a precipitate with picric acid, but this is redissolved on heating to the boiling point.

Ferrocyanic Acid (Hydroferrocyanic Acid) Test. Potassium ferrocyanide and acetic or citric acid mixed in solution set free hydroferrocyanic acid. Does not precipitate peptones.

The following procedure may be used:—*Solution A.*—Citric acid 10 g.; water 100 ml. *Solution B.*—Potassium ferrocyanide 10 g.; water 100 ml. Add 3 ml. of solution A to 4 ml. of the specimen. Mix and add 3 ml. of solution B. In the presence of 0.3% or more of albumin an immediate precipitate is formed. 0.1% is detected on standing for one hour. May also be applied as a ring test.

Further Chemical Tests for Albumin Detection.

Trichloroacetic Acid. A saturated solution, or a crystal, is used in the same manner as in the salicylsulphonic acid test. May precipitate uric acid and nucleoproteins.

Tannin-hydrochloric Acid Test. Mix 5 ml. of the specimen with 5 ml. of warm 1.5% alcoholic tannin solution and add 5 ml. of dilute hydrochloric acid (1 in 3). Turbidity or yellowish precipitate. Interfering substances, such as urates, phosphates and alkaloids, are kept in solution by the acid; resins and alkaloids are redissolved by the alcohol, and peptones by heating.

Heller's Nitric Acid Test. In the bottom of a wide test-tube put a few ml. of concentrated nitric acid, or, preferably, Roberts' reagent (nitric acid 1 part, saturated aqueous solution of magnesium sulphate 5 parts). Slope the tube and allow a few ml. of urine to run down the side. In the presence of albumin an opaque white cloud forms at the plane of contact.

Metaphosphoric Acid, HPO_3 . A fresh solution of a little of this acid is added to the clear filtered urine. A cloud or precipitate indicates presence of albumin.

Millon's Reagent. Dissolve mercury 3 ml. in fuming nitric acid 27 ml. without heat, and dilute the resulting solution with an equal volume of distilled water. With albumin or urea this gives a yellow, then a red coloration on heating.

The directions given above are those of the *B.P.* '32. Many modifications of Millon's reagent have been proposed. The following, for example, is one-third of the strength of the *B.P.* '32 solution in mercury and is possibly the original formula. Dissolve mercury 1 by weight in nitric acid 2 by weight and then dilute with 2 volumes of water.

Asaprol (calcium β -naphtholsulphonate) precipitates albumin, peptone, etc., from acid solution. On boiling, peptone and albumose redissolve, albumin remains.

Albumoses

One may safely regard all proteins in urine which dissolve on heating (after precipitation by a reagent, e.g., salicylsulphonic acid) and reappear on cooling, as albumoses.

Biuret Reaction. Albumin, if present, is removed by 10% trichloroacetic acid solution, and the filtrate tested with the *Biuret Test*:—

In a test-tube place 1 drop of copper sulphate solution (2%), add 5 ml. of urine, and then 5 ml. of sodium hydroxide solution (10%). A rose pink indicates the presence of albumose.

Nickel Reagent. 2 ml. of a solution of 5% nickel sulphate with strong ammonia is added to 4 to 5 ml. of urine made strongly alkaline with potassium hydroxide. Protein present gives a white or greenish-white ring, while an orange yellow ring is given in the presence of albumoses and peptones.

Bence-Jones' Protein. This protein is usually found in the urine of patients suffering from multiple myelomata of the bone. It has been described in other conditions but almost entirely those in which the bone marrow and blood-forming organs are involved. It has been found in cases of secondary deposits of carcinoma in bones, and has been reported in cases of chloroma, leucæmia and lymphosarcoma, but such cases must be extremely rare. About 80% of patients suffering from myelomatosis exhibit the protein in the urine, and the finding of it in urine should always be followed by an extensive X-ray examination of the bones. Bence-Jones' protein has been variously classed as an albumin, a globulin and an albumose but its properties do not exactly coincide with any of these groups. It may be detected by (1) coagulating at 50° or lower, i.e., lower than serum albumin, which coagulates at 75° (on raising to the boiling-point, however, the coagulum dissolves more or less completely, reappearing on cooling). The coagulation point is considerably influenced by the reaction of the specimen. The resolution of the albumose at 100° may not be complete owing to presence of other proteins; (2) precipitation with concentrated hydrochloric acid; (3) precipitation with nitric acid in the cold—on raising to the boiling-point, however, the coagulum dissolves more or less completely, reappearing on cooling; (4) precipitation with potassium ferrocyanide and citric acid (often takes time to develop, differing in this respect from albumin). The hydrochloric acid test is exceedingly sensitive and does not depend on excess of salts. The result is obtainable after very free dilution of the specimen. Some specimens of urine containing a high concentration of albumin give false positive reactions with the hydrochloric acid test. No specimen of urine should be accepted as containing Bence-Jones' protein unless there is some protein portion which can be made to coagulate at 50° or lower, and which is to some extent soluble on boiling.

Amino-acids

Tyrosine (*p*-hydroxyphenylalanine), $\text{OH} \cdot \text{C}_6\text{H}_4 \cdot \text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$, is recognised by its characteristic crystalline appearance, being in shining needles, either in bundles or star form. When tyrosine appears in urine it is usually associated with bile pigment, and the crystals may acquire a yellow colour. The crystals are

soluble in mineral acid, but insoluble in acetic acid, acetone, alcohol and ether.

Further Tests for Tyrosine:—

2 ml. of sulphuric acid mixed with 3 to 5 drops of a solution of aldehyde in twice its volume of alcohol 90%, care being taken that the liquid remains colourless—a few drops added to the suspected liquid produces a gooseberry-red colour. This test is supposed to detect tyrosine in a dilution of up to 1 in 10,000.

Piria states that on adding a few drops of strong sulphuric acid to a little tyrosine in a dish it dissolves with slight reddening on saturating with barium carbonate (after diluting), and on adding to the filtrate neutral ferric chloride solution a violet colour is formed.—Schmidt.

Creatinine. *Syn.* Glycocoll-methylguanidine, $C_4H_7N_3O$. To test for this body in the urine add a little sodium nitroprusside and caustic soda. A red colour develops which fades on boiling the mixture. If a little acetic acid is added to the boiling liquid, prussian blue is produced.

Creatinine, creatine and mucin have a retarding effect on the precipitation of cuprous oxide from Fehling's solution. Urates have an auxiliary effect.

Experiments with diabetic urines showed that creatinine was not increased to any extent even when patients were on a highly nitrogenous diet. Creatine, on the other hand, a substance never found in the normal urine if the diet is free from creatine and creatinine, was always found—even when patients were on a creatine-creatinine-free diet.—*Brit. med. J.*, ii/1910, 1343.

Cystine, $(S \cdot CH_2 \cdot CH \cdot NH_2 \cdot COOH)_2$, is a cleavage product of protein metabolism, apparently loosely bound and easily split off at an early period of the intestinal digestion. Normally it becomes oxidised and hence is unrecognisable, but in cystinuria it is excreted unchanged.

Separation of cystine. Freed from oxalates and phosphates by ammonia and subsequent addition of calcium chloride until this no longer precipitates, add equal volume of acetone and acetic acid in slight excess. Cystine crystallises out in 3 or 4 days. Purify by dissolving in ammonia and reprecipitating.—Mann.

It is occasionally found in urinary deposits as transparent six-sided crystals—insoluble in alcohol but soluble with ease in mineral acids and ammonia. Cystine is insoluble in dilute acetic acid. Uric acid occasionally crystallises in similar form, but gives the murexide reaction; cystine does not.

Leucine (α -aminoisocaproic acid), $(CH_3)_2CH \cdot CH_2 \cdot CH(NH_2)COOH$, occurs as an early result of protein cleavage. There are two isomeric forms of it—*lævo*- and *dextro*-leucine. It occurs in crystalline spheroidal clumps.

Hippuric acid is excreted daily to the extent of about 0.5 to 1 g. on a mixed diet or it may reach 2 or 3 g. on a vegetarian diet. It is formed by the interaction of dehydrated benzoic acid and glycocoll in the system. Protein in the intestines produces amino-acids which are oxidised to benzoic acid. **Glycocoll** is a normal product of metabolism, and by this reaction renders the benzoic acid (*inter alia*) harmless; this occurs, it is thought, in the kidneys.

1 of the free acid in 55,000 of water will change congo red paper to blue, but urine does not cause the change—showing that the hippuric acid present is in the combined condition.

Hippuric Acid Determination. Heat 100 ml. of urine with 10 g. sodium hydroxide in a Kjeldahl flask with reflux condenser for 2½ hours. Then add potassium permanganate 10 g. in small portions and heat gently for 5 to 7 minutes, the liquid remaining at least pink. Cool, and add small pieces of ice, then sodium bisulphite 15 g. Still keeping the liquid cool, add sulphuric acid 1 : 2, *g.s.*, to acidify. Shake out five times with ether. The residue after distilling off the ether is shaken out with chloroform. This dissolves out the benzoic acid formed. Evaporate and weigh. Multiply resulting weight by 1.468 to obtain quantity of hippuric acid.

Quick's Hippuric Acid Test for Liver Function. 5.9 g. of sodium benzoate is dissolved in 30 ml. of water and administered one hour after a breakfast of coffee and toast. The patient is then given half a glass of water.

Immediately after taking the drug the patient urinates and then collects complete hourly specimens for four hours. These are preserved with toluene and the hippuric acid content determined in each specimen. In normal adults the output of benzoic acid, as hippuric acid, is approximately 1 g. or more during the second and third hours, and the total for the four hours is from 3 to 3.5 g. The estimation of the hippuric acid for clinical purposes is done by transferring to a small beaker each hourly specimen, which is measured and is acidified with conc. HCl to congo red. The solution is vigorously stirred until the precipitation of the hippuric acid is complete and then is allowed to stand for one hour at room temperature. The precipitate is filtered off on a small Buchner funnel or a filter plate, washed with a small quantity of cold water and allowed to air dry. The hippuric acid thus obtained is either weighed or titrated with 0.2 normal sodium hydroxide, phenolphthalein being used as indicator. To obtain the total hippuric acid content add to the amount thus obtained the calculated quantity remaining in solution; 100 ml. of urine will dissolve 0.33 g. of hippuric acid. In case any specimen exceeds 125 ml. it should be slightly acidified with acetic acid and concentrated on the water-bath to about 50 ml. before the hippuric acid is precipitated. The synthesis of hippuric acid is strikingly diminished in catarrhal jaundice, in syphilitic cirrhosis and in obstructive jaundice of moderately long duration. The test offers an approximate quantitative measure of liver damage and is a valuable aid in the diagnosis of liver disease. The test cannot be applied in cases of nephritis with nitrogen retention.—*J. Amer. med. Ass.*, ii/1938, 1470. (For other Liver Function Tests, see p. 685 et seq.)

Bile and its Derivatives

The presence of either urobilin, bile pigments or bile salts in the urine indicates some derangement of hepatic functioning. Urobilin is found in small quantities in normal urine, but insufficient to be detected by the ordinary tests. It is formed from urobilinogen, a decomposition product of bilirubin formed by the action of the intestinal bacteria on the bile which passes into the intestine. Under normal conditions a portion of this is absorbed from the intestine and carried to the liver in the portal blood and is there reconverted into bilirubin. When the liver functions are deranged this transformation into bilirubin may be interfered with and urobilinogen reaches the general circulation and is excreted by the kidneys. Thus, tests for urobilin may be positive in the pre-icteric stage of jaundice. Urobilin is increased as the result of excessive blood destruction and in damage to the liver parenchyma. It is nearly or completely absent in obstructive jaundice.

Bile pigments and bile salts usually occur together, although the bile pigment may occur alone. It is seldom necessary, therefore, to test for bile salts. The significance of bile in the urine is similar to that of bile staining of the tissues, and is attributable to obstruction of the outflow of bile from the liver. Small amounts of bile may, however, be found in the urine when the disturbance is not severe enough to produce recognisable jaundice, or in other cases before the jaundice supervenes.

Urobilin. Normally urine contains only traces of urobilin, insufficient to affect its colour, but when there is an excess of pigment the urine is more orange than usual, and a pinkish-yellow tinge may be observed in the lowest layers in a conical receiver.

Spectroscopic Examination. Urine acidified with a little hydrochloric acid shows an absorption band at the junction of the green and blue.

Schlesinger's Test. Urine 10 ml., tincture of iodine 3 to 4 drops. Add to this 10 ml. of alcohol containing about 1 g. of zinc acetate in suspension, shake well and filter. The filtrate by transmitted light will appear yellow or pink. By reflected light it will show a marked green fluorescence. In the above tests any urobilinogen will be converted into urobilin by the hydrochloric acid and iodine respectively.

Bile pigments are simply demonstrated by **Gmelin's Test**. Urine is "layered" over the surface of fuming nitric acid in a test-tube. With normal urine a purple or yellow colour may appear, but in the presence of bile pigment a green colour develops at the junction of the fluids.

Iodine Test. A few drops of tincture of iodine diluted with three times as much water "layered" on to the specimen, and the tube shaken gently, produce a green colour if bile pigment is present.

Chromic Acid Test. A 5% solution added gradually produces a green colour. The following is a modification:—

To 10 ml. of urine add 2 ml. of 0.5% solution of albumin and a few drops of 10% acetic acid; boil and filter. Wash the precipitate with water and to it, on the filter, add 1 drop of a mixture of 4 ml. of 6% potassium dichromate and 1 ml. of 20% sulphuric acid. If bile pigments are present the yellow precipitate turns green or bluish-green. This sensitive reaction is not given by other normal or abnormal urinary pigments.

Ferric Chloride Test. **Obermayer Reagent** prepared by dissolving 0.3 g. of ferric chloride in 100 ml. of concentrated hydrochloric acid. 0.5 ml. of the reagent is added to 5 ml. of urine, and if bile pigment is present the colour at once appears as a deep green—bile may be tested in the same way. For serum, 4 ml. of 95% alcohol is added to 2 ml. of serum and centrifuged, the supernatant liquor withdrawn and 0.5 ml. of reagent added—a green colour will appear in a few seconds.

Fouchet's Test. Place 10 ml. of acid urine in a test-tube, add 5 ml. of 10% barium chloride (if little or no precipitate add 1 or 2 drops of saturated ammonium sulphate solution). Mix and filter. Unfold the filter-paper and spread it on another dry filter-paper. Allow 1 drop of **Fouchet's reagent** (trichloroacetic acid 25 g., distilled water 100 ml., 10% ferric chloride 10 ml.) to fall on to the precipitate. A green or blue colour shows the presence of bilirubin.

Clinical tests for bilirubin in urine.—E. M. Godfried, *Biochem. J.*, 1934, 2057; Hunter, *Canad. med. Ass. J.*, ii/1930, 823.

Bile Salts are shown by **Hay's Test**. Flowers of sulphur is sprinkled on the surface of cold urine in a beaker. The powder floats on the surface of normal urine. Bile salts lower the surface tension and permit the sulphur to sink.

Pettenkofer's Test. Add a few drops of syrup, shake, and then add sulphuric acid—a reddish-violet colour (cf. sodium tauroglycocholate in *Scheme for Recognition of Organic Substances*).

Peptone Test. Peptone in powder 30, salicylic acid 4, acetic acid 30, distilled water 3500. Dissolve and filter. On adding one volume of urine containing bile salts to three volumes of this solution opalescence (or precipitate) appears; it dissolves completely on adding acetic or citric acid, and diminishes, but does not disappear, on boiling.

Blood

Blood in urine may be present in gross amount, when the urine is red, or in small quantity, when it is characteristically "smoky." Mere traces of blood are not discernible by the naked eye. The most sensitive test for blood in urine is the microscopical

examination of the centrifuged deposit for the presence of red cells. Hæmoglobinuria, such as occurs in paroxysmal hæmoglobinuria, where only the hæmoglobin and not the red cells is present, will not of course be detected by this test.

Spectroscopic examination of urine will show characteristic absorption bands of oxyhæmoglobin unless the specimen is old or decomposing when some may have become methæmoglobin also having a characteristic spectrum. Chemical tests for blood all depend upon the hæmoglobin, in conjunction with some oxidising agent, bringing about the production of some coloured oxidation product.

Amidopyrine (pyramidon) Test. To about 5 ml. of urine add 2 drops of acetic acid (33%). On the surface of this mixture in a test-tube carefully layer about 1 ml. of 5% amidopyrine in alcohol. Drop in 5 to 6 drops of 10 vol. hydrogen peroxide. The presence of blood will be shown by the development of a mauve ring at the junction of the two liquids. Iodides in the urine give a false positive and their presence must be excluded.

Benzidine Test. Employ a 1% solution of the base in 90% alcohol or 1% solution in a mixture of equal parts of glacial acetic acid and water. Whichever is used the result is practically identical. There is merely a difference in the shade of blue produced in the presence of blood. To apply the test add to 2 ml. of the benzidine solution about the same quantity of 20 volume hydrogen peroxide. Mix and add 2 ml. of the liquid to be tested. A blue colour forms at once if blood is present. The density of the colour corresponds to the amount of blood. Always conduct a control with normal material alongside. Alternatively, mix 2 ml. of the specimen with a few drops of benzidine solution and layer carefully with ozonic ether. In this case a blue ring is formed.

By the above test 1 of blood in 1000 of liquid is easily detected. Indeed, far smaller amounts, even 1 in 100,000, are stated to have been detected. Diastases, zymases and fruit juices give a similar reaction. A positive reaction does not prove the presence of blood, but a negative proves its absence. The test is less specific if material has been strongly heated.

The Boas benzidine test sometimes gives a positive result when a normal count of erythrocytes is present (up to 1,100,000 red blood cells have been recorded in the 12-hour night urine of normal persons). The following modification of the Boas test will detect 2 to 10 million erythrocytes, or more, in night urines of 150 to 700 ml. having a sp. gr. of 1.015 or more. If the total volume of the night urine is less than 300 ml., 5 ml. is filtered through ordinary filter paper; if the volume is 300 to 500 ml., 10 ml. is filtered; if above 500 ml., 15 ml. is filtered. The filter paper is spread out on a dry filter paper and when the shine of moisture has disappeared 5 or 6 drops of benzidine reagent (25 mg. of benzidine and 0.2 g. of barium peroxide in 5 ml. of 50% acetic acid) are pipetted on to the middle of the filter. A blue colour appearing within 1 to 3 seconds indicates 50 million or more erythrocytes in the total night urine. If the colour appears in from 4 to 30 seconds, 5 to 50 millions are present. A blue colour appearing later than 30 seconds after adding the reagent is considered a negative reaction. The test is less sensitive if the urine is cloudy and does not clear on heating or acidifying with acetic acid, and it cannot be carried out on urines which gelatinise on addition of alkali. On those with a gravity below 1.015 only a negative result is reliable.—K. Larsen, *J. Lab. clin. Med.*, 1937, 22, 935.

Ascorbic acid interferes with the benzidine and guaiacum tests for blood in urine and faeces. The solution to be tested should be boiled, acidified with acetic acid and extracted with ether, which removes the blood pigment but not the ascorbic acid. This also eliminates interference due to iodides and oxidising substances in urine.—J. F. Barrett, *Lancet*, ii/1936, 1214.

The presence of ascorbic acid prevents the formation of the blue colour in the benzidine test for blood. It may be avoided by extracting the urine with ether, evaporating the ethereal extract and testing the residue.—R. Kohn and R. M. Watrous, *J. biol. Chem.*, 1938, 124, 163.

Weber's Guaiacum Test. Make an ether extract as above and add 8 to 10 drops of guaiacum tincture and hydrogen peroxide. Definite blue colour in

2 minutes. For albumin add equal volume of saturated solution of ammonium sulphate, filter, acidify and warm.—R. Coope, *Lancet*, ii/1920, 291.

Ozonic Ether and Guaiacum Test. Add a drop or two of tincture of guaiacum—guaiacum resin 1 in alcohol (90%) *q.s.* to 10—to a small quantity of the urine, shake and “layer” ozonic ether on to the mixture. A blue colour at once, or on standing, indicates presence of blood—iodine in the urine also gives this colour (e.g., if patient has been treated with iodides). Further, pus gives it with guaiacum tincture alone, the colour disappearing on heating.

Modified Guaiacum Test using Sodium Perborate. To about 5 ml. of the liquid add 1 to 5 drops of fresh saturated alcoholic solution of guaiacum resin, then about 1 g. of sodium perborate and about 10 ml. of 30% acetic acid, shake the mixture once and pour alcohol carefully into the tube to form a separate layer,—a blue or blue-green colour at the junction in 5 minutes will be formed, or green if only a trace. The guaiacum resin used must show a brown, not a greenish fractured surface.

This test is said to indicate 0.02 g. of blood per litre, i.e., 1 in 50,000. It is about five times as delicate as the ozonic ether test. A green colour should be disregarded since a blank test gives a green. Fresh solution of guaiacum is stated to have no advantage over seven months' old simple tincture of guaiacum.

Paraphenylenediamine Test. Pour into a test-tube 1 ml. of alcoholic 1% paraphenylenediamine, 5 ml. of 27% formic acid and 3 drops of 6% hydrogen peroxide; add 3 ml. of urine, shake, and allow to stand; a green colour indicates the presence of blood. The colour is produced with 3 drops of a solution of 0.5 ml. of blood in 2000 ml. of normal saline.—V. I. Zarauz, *per J. Amer. pharm. Ass., pharm. Abstr.*, 1942, 17.

Calculi

Urinary Calculi. The size and shape of urinary calculi depend on the region of the urinary tract in which they form. Their chemical composition depends on the character of the urine in which they form. Since the urine may frequently change its character during the slow growth of the stone the end result is usually a laminated calculus with layers of different colour, consistence and chemical composition. There may or may not be an organic nucleus to the calculus. Changes in the reaction of the urine are primarily responsible for the progressive growth of the calculus, increasing acidity causing uric acid to deposit and increasing alkalinity causing deposition of phosphates and urates. It is probable that many calculi centre round a primary uric acid infarct of the tubules of the kidney. The chief secondary changes produced by a calculus are hæmorrhage, infection, and the deposition of organic elements round the stone. The commonest varieties of urinary calculi are the calcium oxalate, uric acid, ammonium urate and phosphatic calculi.

Calcium oxalate stones are extremely hard, usually round, and distinguished by their dark brown colour and by their rough “mulberry-like” exterior. They are often formed around a nucleus of uric acid or urates. The irritation caused by the rough surface leads to the deposition of carbonates and phosphates which may fill up the depressions and smooth off the surfaces.

Phosphatic calculi have a rough white crumbly surface. They form when the bladder is inflamed and consist chiefly of ammonio-magnesium phosphate and calcium phosphate.

Uric acid calculi are smooth and hard, like pebbles. The centre may contain a granule of ammonium urate round which fine delicate yellow laminæ are regularly laid down. A pure uric acid calculus may not show by X-rays because its permeability is practically the same as surrounding soft tissues but mixed calculi composed of uric acid with calcium phosphate or oxalate may be recognised radiologically.

Cystine. Calculi containing cystine are usually fairly pure, but occasionally they are found with a superficial phosphate coat. They are rare.

Xanthine as a urinary calculus is extremely rare.

Chemical Examination of Urinary Calculi.

If possible a representative sample should be analysed either by examination of half a powdered calculus or by examination of the individual layers. Where the preservation of the stone is important it is best to use the powder obtained from a boring to the centre.

Preliminary Examination. Heat a small portion of the powder on platinum foil. This will show if the bulk of the calculus is chiefly organic or contains a high proportion of ammonium salt. The presence of cystine will be shown by a blue flame and a pronounced mercaptan-like odour. All calculi contain a proportion of organic matter.

Platinum Wire Test. Heat a small portion of the powder in a platinum wire loop in a burner flame and after moistening with a drop of concentrated HCl again heat in the flame. Calcium which has been present in the original powder as oxalate or carbonate will yield a brick-red flame. Calcium phosphate will not give a flame but when the powder is first heated in the platinum wire the calcium oxide from calcium oxalate and calcium carbonate will give a limelight effect. Calcium phosphate will remain as an infusible white powder and ammonio-magnesium phosphate will melt and run into a bead.

To decide if a calculus consists chiefly of uric acid and phosphate or oxalate the following solubility test is useful. Boil a small amount of the powder with 50% HCl. This will dissolve all except uric acid. The insoluble uric acid will be found to be readily soluble in a solution of lithium carbonate. Confirm by applying murexide test to the original powder. Flocculent organic debris should not be mistaken for the insoluble uric acid. To the filtrate from the above add concentrated ammonia until there is a slight excess. Any precipitate will be due either to phosphate or oxalate. Add glacial acetic acid until there is slight excess. Phosphate will dissolve, calcium oxalate will be insoluble. Confirm calcium oxalate by flame test and phosphates by molybdate reaction. Cystine would be precipitated from the ammonia solution by the acetic acid. Confirm by lead test or by seeing characteristic hexagonal crystals microscopically.

Murexide test for presence of uric acid. Heat a pinch of the original powder in an evaporating dish with 3 or 4 drops of concentrated nitric acid. A red colour develops. A drop of 40% sodium hydroxide will give a mauve colour.

Molybdate test for phosphates. Warm a pinch of the original powder with a few drops of concentrated nitric acid in a test-tube, add 3 to 4 ml. of 10% ammonium molybdate and warm. A canary yellow colour and precipitate will form if phosphates are present.

Lead test for cystine. Boil a pinch of powder with 3 to 4 drops of solution of lead acetate and 5 to 6 drops of 20% sodium hydroxide. A black precipitate of lead sulphide will form if cystine is present. Ammonia may be detected in the calculi by boiling a pinch of the powder with 20% sodium hydroxide. The ammonia may be recognised by its odour or by litmus paper.

Biliary Calculi (Gall Stones). Concretions forming in the gall bladder and bile ducts. Large gall stones generally consist almost entirely of cholesterol with some bile pigments. Smaller stones contain a higher proportion of bile pigments and calcium, while very small calculi composed of bile pigment only occur.

Calculi found in the common bile duct often contain a high proportion of fatty acids. Pigmented gall stones commonly contain a small amount of copper.

Casts

The presence of casts may be an important indication of renal disease. The centrifuged deposit is examined both with the $\frac{2}{3}$ and later with the $\frac{1}{6}$ inch objective. Casts have a sharp outline. Their significance depends on their character.

Cellular casts may be composed of red blood corpuscles, pus cells or the mononuclear cells of renal epithelium, and indicate acute nephritis. In chronic parenchymatous nephritis and in acute nephritis after the first few days of the disease the casts are usually granular, and represent a later stage of the cellular casts which have undergone a granular degeneration.

Hyaline casts, with structureless contents, may be found in any form of nephritis but are commonest in chronic interstitial nephritis and hyperpiesia. A few hyaline casts may be present in the urine of healthy people.

Cylindroids are pale ribbon-like bodies with tapering ends which are found in inflammations of the urinary tract.

Chlorides

Instead of evaporating and incinerating with ammonium nitrate, oxidise the organic matter contained in 10 to 20 ml. of urine by warming with 3 ml. of sulphuric acid and a slight excess of potassium permanganate solution. The addition of a few drops of hydrogen peroxide will then cause the precipitated manganese dioxide to dissolve giving a clear solution. To this add a few ml. of nitric acid and a measured excess of N/10 silver nitrate solution, boil and cool. After adding 1 or 2 ml. of ferric alum solution to the mixture, titrate the excess of silver with N/10 ammonium thiocyanate.

Alternative Method. 5 ml. of sample is shaken with 0.1 g. of decolorising carbon and filtered. 1 ml. is diluted to 10 to 15 ml. with water, 1 ml. of 0.01% aqueous solution of eosin and 2 ml. of a 0.005% solution of dichlorofluorescein in dilute aqueous alkali are added and the whole titrated with a solution of silver nitrate containing 5.812 g. per litre, until the precipitate becomes magenta.—C. F. M. Rose, *Biochem. J.*, 1936, 1140.

Chyle

Chyle gives to urine a milky appearance due to the presence of an emulsion of fat which sometimes separates on standing. The urine is acid, with sp. gr. usually between 1.015 and 1.020; it is coagulated with nitric acid, contains 0.6 to 0.9% of protein, 0.8 to 1.8% of fat, about 1.5 to 2% of urea, and no sugar. Often the fat emulsion is so fine that no creaming occurs on standing. The fat may be removed by shaking with ether but the shaking must be prolonged. Chyluria is usually due to rupture of lymphatics in the bladder wall due to an obstructive condition in the pelvis. Sometimes the condition diagnosed as chyluria is really due to chyle passing *per vaginam* becoming mixed with the urine.

Sudan III Test of True Chyluria. 100 mg. of Sudan III is given in 10 g. of butter. Normally no dye will appear in the urine. In chyluria the urine will be pink owing to the dye remaining attached to the fat excreted in the urine.

Glucose

The absorption, circulation and utilisation of sugar depend on the harmonious activity of many different organs of the body and glycosuria may result from the breakdown of this co-operative activity at different points. It is most important to remember that glycosuria does not necessarily indicate diabetes. It is only after the exclusion of the less serious causes of glycosuria that a diagnosis of diabetes is justified. The bulk of carbohydrate is absorbed from the intestine in the form of glucose. This travels to the liver via the portal circulation. Part is stored in the liver and part in the muscles as glycogen. The speed and efficiency of storage is reflected in the blood sugar and is controlled by the insulin of the body. A lack of insulin means delayed or partial storage and a correspondingly high blood sugar. When the blood sugar reaches a certain level, sugar will appear in the urine.

Alimentary glycosuria results when sugar is absorbed from the intestine more quickly than it can be stored, so that the blood sugar rises and the leak point is passed. This is not a common occurrence, and experiments have shown that it is difficult to produce alimentary glycosuria in healthy individuals even after the consumption of enormous quantities of sugar.

Renal glycosuria results when the leak point of the kidney is set at a lower figure than normal. Thus some individuals excrete sugar in the urine even when the blood sugar is only 140 mg. per 100 ml. In such people glycosuria appears after a carbohydrate meal when the blood sugar is likely to rise. Renal glycosuria is an innocent condition, but it is advisable to keep such patients under observation.

Endocrine glycosuria occurs in hyperthyroidism, pituitary tumours and after injections of adrenaline. The hormones here involved probably act by stimulating the liver to break down glycogen, with the result that hyperglycæmia and glycosuria result.

Injuries to the floor of the fourth ventricle cause sugar to appear in the urine, and this has been described as **nervous glycosuria**. The probable explanation is that injuries to this region of the brain cause a stimulus to the suprarenals with an increase in the adrenaline in the blood, and therefore an adrenaline glycosuria.

True diabetes results from the inability of the tissues to utilise carbohydrate, owing to the absence of insulin provided by the pancreas. This inability to utilise carbohydrate is interpreted by the body as a stimulus for the production of more glucose so that all available carbohydrate is poured into the blood, producing a hyperglycæmia. Since the tissues cannot utilise the sugar it is excreted in the urine.

These different forms of glycosuria can be distinguished by estimations of the blood sugar and observation of the glucose tolerance test.

Tests for Sugar in Urine.

Benedict's tests for sugar in urine are those most commonly used. They are simpler and avoid the fallacies of Fehling's test.

Benedict's Quantitative (Modified Fehling) Test. Copper sulphate 18 g., sodium carbonate cryst. 200 g., sodium citrate 200 g., potassium thiocyanate 125 g., 5% potassium ferrocyanide solution 5 ml., water to 1 litre. The test is as Fehling's—the end-point being disappearance of the blue colour.

To conduct the test 25 ml. of the reagent is measured into a small flask and about 4 g. of anhydrous sodium carbonate added and the solution brought to the boil. Urine is then run in slowly from a burette until the reagent turns from clear blue to an opalescent bluish-white colour, when the additions are made more carefully until the colour disappears. This gives a rough indication of the amount of sugar in the urine and the titration is repeated after first diluting the urine so that about 10 ml. will reduce the reagent. 25 ml. of the reagent is reduced by 0.05 g. of glucose. This quantity is therefore in the volume of urine reducing 25 ml. of the reagent.

Benedict's Qualitative Test. Dissolve with heat sodium (or potassium) citrate, 173 g., and 100 g. of anhydrous (or 270 g. cryst.) sodium carbonate in water, about 700 ml. Dissolve separately pure crystallised copper sulphate, 17.3 g., in water about 100 ml. Cool to room temperature, pour the second into the first solution slowly with stirring and make up to 1000 ml. with distilled water.

The patient can employ the test himself. 5 ml. is boiled with 8 drops of urine for two minutes and cooled. If glucose is present the colour changes to an opalescent green, or, in the case of a large quantity of sugar, to an opaque red. Benedict's reagent is reduced by glucose, lævulose, lactose, pentose and homogentisic acid, but not by uric acid and creatinine. To confirm and distinguish glucose employ the fermentation test (*see p. 654*).

Modification of the Benedict Test. The following is a simple, rapid and fairly accurate method for use by patients or doctors when it is difficult or impossible to carry out the original Benedict test: 2 ml. of Benedict's solution are placed in a test-tube with a 2 ml. mark and to this are added 6 drops of urine to be examined. Shake well, boil over a free flame for a few seconds and allow to stand at room temperature for 30 seconds or more. The results obtained may be interpreted as follows: a translucent green discoloration indicates about 0.05% of glucose; an opaque green, 0.1%; yellowish green 0.5%; dusky brown 1%; bright orange red 2%. In urines containing more than 1% one part of urine is mixed with 9 parts of water and 6 drops of diluted urine are used to 2 ml. of Benedict's solution. The readings are then as follows: opaque green indicates 2% glucose in the undiluted urine; green with a tinge of yellow 4%; yellow with a tinge of green 8% and light orange 12%. It is important that a standard dropper should be used and that this should be rinsed well after using, and in making dilutions of urine containing a high percentage of sugar the millilitre and not the drop should be employed as a standard of measurement.—S. Gold, *Canad. med. Ass. J.*, 1941, 445.

Fehling's Test. Glucose, being an aldehyde, has a strong reducing action. In the test the alkaline glucose-cupric oxide, when heated, causes deposition of cuprous oxide. 1 molecule of

glucose reduces practically 5 molecules of cupric oxide. In making use of Fehling's solution it is important when looking for small quantities of sugar to dilute the urine to about sp. gr. 1.015. Mix with an equal volume of mixed Fehling's solution. Boil for a few seconds—if no precipitate appears within two minutes there is no sugar of pathological import. Great care, however, should be taken not to confuse with reducing substances other than glucose.

Fehling's Solution is prepared in two solutions:—No. 1. Copper sulphate 34.64 g., sulphuric acid 0.5 ml., distilled water to 500 ml.

No. 2. Sodium hydroxide 77 g., sodium potassium tartrate 176 g., distilled water to 500 ml.

Mix equal volumes when required. Of this, 10 ml. will be decolorised and reduced by 0.05 g. (or 53 minims by $\frac{1}{4}$ grain) of glucose or diabetic sugar in solution, with precipitation of yellowish-red cuprous oxide when the two are boiled together. No. 2 solution should not be kept in a very cold place or it may crystallise. By keeping the copper solution separate from the alkaline solution the test is prevented from becoming erroneously sensitive. A little calcium carbonate or barium sulphate greatly assists the deposition of the cuprous oxide and enables the colour of the supernatant liquid to be more easily seen.

Fehling's is reduced by dextrose, lævulose, mannitose, milk sugar, galactose, arabinose, aldehyde, formaldehyde, chloral, chloroform, creatinine, valeraldehyde, resorcinol, pyrogallol, gallotannic acid, arsenic trioxide and similar reducing bodies, glycosides, and acetone; also by glycuronic acid, uric acid, pyrocatechin, hydroquinone and salicylic acid compounds; these may be removed by simple repeated filtration through animal charcoal. None of these bodies ferments or gives osazone crystals. Trichloroacetic acid does not reduce Fehling itself, but tends to inhibit the reduction by sugars, so that quantitative results cannot be obtained in its presence.

An orange precipitate formed when hot urine is mixed with hot Fehling's solution without reboiling affords almost conclusive evidence of the presence of a hexose monosaccharide such as glucose or lævulose.

An orange precipitate formed on boiling is sometimes due to the presence of a compound glycuronate.

To make certain of glucose the urine must contain a + rotatory reducing substance, fermented by yeast (both glucose and lævulose are), it must yield an osazone of the correct crystalline form, melting at slightly above 200° (both glucose and lævulose give this) and finally it must yield no osazone in case of glucose with methylphenylhydrazine, which, with lævulose, yields one melting at 150°.

Lloyd's Reagent (ALUMINIUM SILICATE) removes interfering substances. To 5 ml. of urine add 5 ml. of N/10 sulphuric acid and 10 ml. of water. Add 1.5 g. of Lloyd's alkaloidal reagent and shake gently for 2 minutes. This removes most of the colouring matters, uric acid, creatine, creatinine, yet unlike charcoals, does not take away the sugar. (It is not necessary to remove every trace of creatinine). Filter. 2 ml. used for the usual colorimetric sugar determination. Shaking should not be continued for longer than 2 minutes, as acid gradually dissolves the reagent—the dissolved aluminate does not, however, interfere. With more dilute urines, use 10 to 15 ml. For total sugars invert 10 ml. of above filtrate by heating in boiling water for 75 minutes with 1 ml. of 10% hydrochloric acid. Cool, neutralise to phenolphthalein, dilute to 20 ml., add a small pinch of Lloyd's reagent, shake, and immediately filter. Take 2 ml. for determination.

Fehling's is not reduced by mannite, dulcitol, sucrose, inositol, cellulose, dextrin, arabin, alcohol, glycerin, phenol, benzaldehyde, salicyl aldehyde, acetic, lactic, oxalic, succinic, tartaric, citric, gallic, saccharic, mucic, gluconic, benzoic, salicylic and sulphurous acids, and alkaloids.

Trommer's Test. To 5 ml. of urine add $\frac{1}{2}$ vol. of 15% sodium hydroxide and then 1 ml. of 10% copper sulphate solution. A red or yellow precipitate appears on standing in the cold a few hours, more rapidly on boiling. On heating, much of the cupric hydroxide may remain undissolved—an excess of alkali is necessary as in the case of Fehling's solution (or less copper solution can be used). Fehling's test has superseded Trommer's. They are employed in the same manner. Trommer's test may be interfered with by creatinine. It is important to add the alkali before the copper solution.

Barfoed's Test. This is not a specific test for glucose, but serves to detect monosaccharides. Barfoed's solution is neutral copper acetate 13.3, acetic acid solution (1%) 200. A glucose solution warmed with a small quantity of this precipitates cuprous oxide. The presence of chlorides causes the formation of a green precipitate.

An improved form contains copper acetate 50, sodium acetate 50, glacial acetic acid 5, water to 1000. With this, reduction is obtained with 0.1% dextrose solutions on merely heating to boiling, while 1% solutions of maltose or lactose do not show reduction under similar conditions.—*J. chem. Soc. Abstr.*, ii/1921, 525.

Nylander's Test. To 5 ml. of urine in a test-tube add 1 ml. of Nylander's reagent (bismuth subnitrate 2 g., Rochelle salt 4 g., 8% sodium hydroxide 100 ml.), and boil for two minutes. In the presence of glucose a black precipitate will appear. Albumin, if present in the urine, must be removed before applying, since it gives a similar precipitate. The test is not affected by the presence of uric acid or creatinine.

Cramer's Mercury Test. Dissolve mercuric oxide (red or yellow) 0.4, with potassium iodide 6, in water 100 and adjust the alkalinity of the solution so that 10 ml. is neutralised to phenolphthalein by 2.5 ml. of N/10 acid.

To use the test heat 3 ml. of the solution to boiling, add 0.3 ml. of urine and boil again. On removing from flame the mixture darkens if sugar is present. Metallic mercury settles ultimately. Glucose, lactose, maltose, xylose and arabinose give the reaction, but not cane sugar. The test is quite sensitive. The deposit of metallic mercury is of greyish colour.

Picric Acid Test (Johnson's or Braun's Test). This has been suggested as a test for glucose in urine, since a solution of this sugar, if boiled with picric acid and solution of potash, reduces the yellow picric acid to the deep red picramic acid, forming potassium picramate, the depth of colour depending on the amount of sugar.

Safranin Solution Test. One volume of 1 in 1000 safranin solution with one of urine and one of solution of potash is heated to boiling, avoiding agitation. If the urine contains sugar to the extent of 0.1% the liquid will be decolorised. (On cooling, the colour may return in proportion to the amount of sugar present.) Each additional volume of the safranin solution that may be decolorised represents roughly 0.1% of sugar. Safranin solution is unaffected by creatine, creatinine, uric acid and urates.

Phosphomolybdic Acid Test. For estimating normal urinary sugar. Toluene is useful to preserve. 1 ml. of specimen is precipitated by 2 ml. of phosphotungstic acid reagent made of 2% phosphotungstic acid in 5% sulphuric acid. A dense precipitate is formed. After thoroughly shaking, dilute to 10 ml. by adding 7 ml. of distilled water; shake and filter. This filtrate is clear and free from creatinine and other interfering substances. 1 ml. of the liquid is heated 6 minutes in a boiling water-bath with 2 ml. of alkaline copper solution. Remove and add phosphomolybdic acid solution. Presence of sugar is shown by a deep blue colour. This can be made colorimetric by comparison with a glucose solution. The amount normally present is very constant. It exceeds 1 g. a day on ordinary mixed diet, and the percentage in the urine corresponds curiously closely with that regarded as normal in blood, namely, 0.08 to 0.1%.—*R. L. Mackenzie-Wallis, Lancet*, ii/1921, 1003.

Dinitrosalicylic Acid Test. For colorimetric determination of sugar. To 10 g. of phenol add 22 ml. of 10% sodium hydroxide and make up to 100 ml. Add 69 ml. of this solution to 6.9 g. of sodium bisulphite and mix with 300 ml. of 4.5% sodium hydroxide, 255 g. of Rochelle salt and 880 ml. of 1% dinitrosalicylic acid solution. To 1 ml. of urine add 3 ml. of reagent, heat for 5 minutes in boiling water, cool and dilute to 25 ml. Compare with standards containing 1, 0.5, and 0.25 mg. of glucose. Concentrated urines containing

over 0.18%, and dilute urines containing over 0.12% of sugar, can be considered abnormal.—J. B. Sumner, *J. biol. Chem.*, 1925, 65, 393.

Fermentation Test. A useful confirmatory test. Prior to conducting, determine the specific gravity of the urine as exactly as possible. Then fill a Doremus tube completely with the specimen; place a little fresh yeast in the bend; keep in a moderately warm position for 24 hours. If sugar is present, carbon dioxide will be produced, and the gravity of the urine will fall—each degree of density lost being equivalent approximately to 1 grain of glucose per ounce. A decrease of 0.001 in the sp. gr. corresponds to 0.23% of sugar. When the sugar content is not less than 0.4 to 0.5%, and the readings are carefully taken at the same temperature, the method gives fairly exact results.

Fungi in Relation to Human Pathology. The "Yeast Method" of detecting glucose in urine is not specific. Ordinary samples of baker's yeast will ferment lævulose, maltose, galactose, saccharose, lactose, and other carbon compounds in addition to glucose. (This difficulty can be overcome by washing and filtering the yeast and by putting up suitable control tests against known solutions of lactose which is the commonest cause of doubt.—Archer). A germ should be used which ferments glucose only, e.g., *Monilia balcanica* Castellani, or, failing this, *Monilia Krusei* Castellani, which ferments glucose and lævulose only.

A mycologic method described especially useful for identification of maltose and galactose.—A. Castellani and F. E. Taylor, *J. trop. Med. (Hyg.)*, 1926, 209. See also A. Castellani, *Lancet*, i/1920, 847, 895, and P. Pietra, *J. trop. Med. (Hyg.)*, 1927, 182.

Carbohydrate Test. The char is most distinctive of a saccharine urine.—J. Barker Smith, *Pharm. J.*, ii/1924, 309; i/1925, 100.

Galactose Tolerance Test for Liver Function. The patient is allowed no food overnight or at breakfast time. In the morning, after emptying the bladder, he is given 40 g. of galactose in 250 or 300 ml. of water. The urine is then collected hourly for 5 hours, each specimen being tested qualitatively for sugar. The positive samples are mixed and the sugar estimated by Benedict's method (25 ml. of Benedict's quantitative reagent are reduced by 0.054 g. of galactose) and the 5 hours' output of sugar calculated. In patients with glycosuria the glucose may be removed, without appreciable loss of galactose, by fermentation. Make a 10% suspension of thoroughly washed yeast. To 7.7 parts of this add 1 part of urine; keep for 45 minutes at room temperature and determine the sugar remaining, which is all galactose, by Benedict's method.

Any excretion in excess of 3 g. shows decreased tolerance. The chief value of the test is in differentiating obstructive from non-obstructive jaundice. Positive results are common in the latter but rare in the former.

Lactose

Lactose seldom occurs in the urine except in nursing mothers, or soon after weaning, and its importance lies in its differentiation from glucose; lactose does not ferment with pure yeast and gives a characteristic osazone.

Osazone Test. Phenylhydrazine hydrochloride, as much as will cover a sixpence, is dissolved with twice its quantity of sodium acetate in about 1 ml. of glacial acetic acid. To this is added 10 ml. of urine. If the solution is not clear after well mixing it should be filtered. The filtrate is placed in a tube in a boiling water-bath for 30 minutes. The water-bath should then be allowed to cool with the tube still in it so that the very slow cooling will help the formation of characteristic crystals.

Lævulose

Lævulose reduces Fehling's solution, ferments with yeast and forms an osazone with phenylhydrazine like glucosazone. Occasionally found in urine alone—more commonly with dextrose.

Pseudo-lævulose of diabetic and other urines. True lævulosuria or fructosuria may be met with, but apparently it is

rare. The lævorotatory body is in reality the ketonic acid, isoglycuronic acid, which is differentiated from lævulose by Borchardt's test—the acid is precipitated from an acid solution on saturation with lead acetate—and the melting-point of the parabromphenylosazone. Specimens from 30 cases of so-called lævulosuria and 50 of diabetes, in which a lævorotatory substance was present along with dextrose, were examined and in none could any true lævulose be found.

Borchardt's Modification of Seliwanoff's Test consists in treating the specimen with hydrochloric acid and resorcinol, making alkaline with sodium carbonate and extracting with ethyl acetate. With *plant* lævulose the extractive is red in colour, but with urines giving the ordinary Seliwanoff reaction the watery solution retains the pigment and the extract is yellow.

Seliwanoff's Reaction for Lævulose. On warming a solution of resorcinol in 1 part of concentrated hydrochloric acid and 2 parts of water with lævulose, an intense red coloration is formed and gradually a dark precipitate soluble in alcohol with production of a red colour. Glucose, lactose, maltose and pentoses do not give this colour. Glucose will give a positive reaction if the boiling is prolonged.

Seliwanoff's Reaction for Cane Sugar. The test applied exactly as above gives only a very faint pink on warming. Takes some minutes to form. Using strong hydrochloric acid, the reaction for both is the same. The precipitate in both cases is soluble in alcohol.

Salicyluric Acid. This substance occurs in the urine of a person taking salicylate or a compound of salicylic acid, e.g., aspirin, and gives a reduction of the ordinary reagents such as Benedict's or Fehling's. It is not fermented by yeast and does not form an osazone. The urine in these cases always contains much salicylate and gives a deep purple colour with ferric chloride solution. A reduction due to salicyluric acid and not glucose should always be suspected in urine giving a marked reaction with ferric chloride for salicylates.

Pentose

Bial's Test (P.G. VI). Orcin 1 g. in 500 ml. of concentrated hydrochloric acid containing 25 drops of ferric chloride solution.

Method of Use. To 10 parts of reagent add 1 part of urine and bring just to the boil. Allow to stand until cold. Pentoses yield a green colour and a bluish-green precipitate. Glycuronates will give the same reaction only after thorough boiling. After cooling, shake out with 2 parts of amyl alcohol. The amyl alcohol extract will be green, and spectroscopic examination will show a band between the C and D lines overlapping the D line. A second band nearer the red band of the spectrum is not of any significance. A green colour alone is not proof of the presence of a pentose.

Alkaptonuria is due to the presence of dioxyphenyl acetic acid (homogentisic acid).

Alkapton urine is normal in colour when freshly passed but rapidly darkens on exposure to air and light. The oxidation is hastened by alkali. It reduces Fehling's solution and also solution of silver nitrate in the cold.

Ferric Chloride Test for alkaptonuria. 5% solution of ferric chloride is added drop by drop to 10 ml. of urine in a test-tube. With each drop a fleeting blue colour is given if homogentisic acid be present.

Pentosuria and alkaptonuria are fully described in *Inborn Errors of Metabolism*, by Sir A. E. Garrod.

Indican

Indican, potassium indoxyl sulphate, is excreted in pathologically abnormal quantities in the urine when excessive putrefaction occurs in the intestine.

Indicanuria is most commonly found in intestinal obstruction and severe intestinal inflammations. It may be present in chronic gastritis, gastric cancer and diminished hydrochloric acid secretion.

Decomposition of exudates anywhere in the body as in empyema, bronchiectasis and large tuberculous cavities may cause indicanuria.

Small amounts of indican in urine are of no pathological significance. The taking of milk in fairly large quantities will always result in a positive test for indican in the urine.

It is tested for as follows:—To a test-tube one-third full of urine is added an equal quantity of Obermayer's reagent, and a few millilitres of chloroform. (OBERMAYER'S REAGENT consists of ferric chloride 2, hydrochloric acid 1000. This makes a yellow fuming liquid which keeps well.) Invert the test-tube a few times to mix. If indican is present in excess the chloroform will assume an indigo blue colour.

Urine of patients taking potassium iodide may give the colour, and this may obscure a strong indican reaction. This can be removed by shaking with a little sodium thiosulphate, leaving the blue of indican. Occasionally, owing to slow oxidation, indigo red will appear instead of indigo blue. This gives a colour much like that due to iodides but it does not disappear when treated with sodium thiosulphate. Bile pigments which interfere with the test must be removed by precipitating with normal lead acetate solution and filtering.

Phosphates

Phosphoric acid exists in urine as salts of sodium, potassium, ammonium, calcium and magnesium. Excretion of phosphates is very variable; the average for 24 hours is 2.5 g. as P_2O_5 . The organic phosphorus content of urine is very small, being about 2% of the total. Estimation of urinary phosphates is of no value except as part of a complete metabolism experiment where intake of phosphorus is known. It is usually estimated by titration with standard uranium acetate, potassium ferrocyanide being used as an external indicator.

Deposition of earthy phosphates in urine is usually due to change of reaction rather than a true increase of excretion. Inorganic phosphate content of blood plasma 2 to 4 mg. per 100 ml.; children, 4 to 5 mg. Decreased in active rickets and by insulin. Increased in chronic nephritis, healing of major fractures, diabetic coma.

Porphyrins

Two porphyrins may occur in urine, uroporphyrin and coproporphyrin. The amounts of porphyrins excreted in health are so minute, that for clinical purposes it may be accepted that they do not occur in normal urine. When present in abnormal quantities, porphyrins impart a port-wine colour to the urine. Porphyrinuria may occur in patients susceptible to certain hypnotics, such as sulphonal and trional, and to antipyretics, such as acetanilide, phenacetin, phenazone, amidopyrin, aspirin, etc., or may arise from the long-continued use of such drugs; it may also result from the use of the sulphonamide group of drugs. In addition to the foregoing it is a frequent accompaniment of T.N.T. poisoning in workers engaged in the manufacture of explosives, and a careful watch should be kept for this early symptom of poisoning.

When urine containing porphyrin is examined with filtered ultra-violet rays it shows a pink fluorescence.

Tests for Porphyrinuria in T.N.T. Poisoning.

Webster's Test. 12.5 ml. of the urine mixed with an equal volume of sulphuric acid (20 : 100) and shaken with 10 ml. of ether. The acid liquor is rejected and the ether washed once with 25 ml. of water. Then treat this ether solution with 5 ml. of alcoholic potash solution (4 or 5 : 100). When T.N.T. is present a purple colour is at once formed varying in intensity. Care must be taken to distinguish between absorbed T.N.T. and accidental contamination.

Improved Webster Test. Place 50 ml. of urine in a 400 ml. flat-bottomed glass beaker and add 50 ml. of sulphuric acid (dilute) 20% by volume. Bring

to the boil and allow to boil for not more than one minute. Allow to cool, and examine 12 hours later. If negative the solution will be quite clear, lemon-coloured, pale brown or reddish-brown. Occasionally, when the solution is reddish-brown the merest trace of a fine granular deposit of a purplish-red colour may be found in distinct quantity on the bottom of the glass. If positive, the solution will be reddish-brown and a fine granular deposit of a purplish-red colour will be found in distinct quantity on the bottom of the beaker. For purposes of day-to-day comparison the positive (toxic) urines may be classified and recorded as follows:—++ (strongly positive): solution, deep reddish-brown: deposit, abundant, dark purple. + (positive): solution, reddish-brown: deposit considerable, purplish-red. ± (weakly positive): solution, reddish-brown (lighter than +): deposit, moderate, purplish-red. It is suggested that T.N.T. workers giving ++ and + should be removed from this work and those giving ± should be watched and tested again.—A. M. Kennedy and J. Ingham, *Brit. med. J.*, i/1942, 490.

Pus

On finding pus in the urine it should be examined bacteriologically to ascertain the nature of the infecting agent. Radiological examinations may be necessary also to rule out the question of calculus.

In women a catheter specimen of urine is required, but in men it is possible to obtain a suitable specimen without catheterisation if the glans penis is carefully cleaned and the patient instructed to pass the first portion of urine into an ordinary receiver and the next portion into a sterile wide-mouthed bottle. The specimen must be despatched to the laboratory immediately after collection.

Microscopic examination is essential when pus cells are found in the centrifuged deposit: a count should be made of the number of cells per cubic millimetre. A drop of the fresh uncentrifuged urine is examined in a counting chamber. A few leucocytes may be present in the urine in health, and as the result of several thousand tests C. E. Dukes has shown that 0 to 10 leucocytes may be present per cubic millimetre in the urine of healthy people; he suggests as a definition of pus "more than 100 leucocytes per cubic millimetre." The intermediate zone between 10 and 100 is described as the zone of excess of leucocytes. Degrees of pyuria have been defined by Dukes as follows:—100-1000 pus, 1000-10,000 pus +, and 10,000-100,000 pus ++.

Mucus threads are sometimes found in the urine of male patients who have had gonorrhœa several years previously. These appear as a long thread-like process of mucus uniting chains of pus cells. They are common after prostatic massage and may be present only in the first morning specimen.

Cholesterolin is rarely found. It is usually derived from a collection of pus that has been retained in a cavity for some time, ultimately discharging into the urine.

To separate cholesterolin, extract the specimen with alcohol-free ether. Purify the residue on evaporation, by dissolving in strong alcoholic potash, evaporating, extracting again with ether, and this again with boiling alcohol—rhombic plates.

Chloroformic solution of cholesterolin with sulphuric acid gives a red to purple colour. An alcoholic solution so treated gives red to blue.

Urea

The average content of urea in urine is 2.5 to 3%, or about (in health) 500 grains (33 g.) per diem; it may range between 15 g. and 40 g. The majority of methods are based on the decomposition of urea into nitrogen, carbon dioxide, and water when treated with sodium hypobromite. The carbon dioxide is absorbed by the excess of alkali present, and the nitrogen can be measured,

from which, on reference to tables, the percentage can be found—theoretically 1 ml. of nitrogen at $0^{\circ}=0.0027$ g. approximately of urea. In the process about 8% of the total nitrogen is suppressed, but the increase in volume of the gas due to the room temperature (taken as 18°) and the vapour tension (the gas being measured moist) has been found in practice to compensate almost exactly for this loss.

The average urea content in urine was found to be 1.6% in the case of patients under observation in hospital. When the kidneys are damaged the concentration falls below that figure. That the specific gravity of the urine is consistently low in advanced granular kidney disease is a well recognised fact. Salt retention is a characteristic of the oedematous or tubal type of nephritis just as urea retention is characteristic of the chronic interstitial or cirrhotic type.—C. R. Box, *Brit. med. J.*, i/1920, 356.

Sodium Hypobromite Method. Sodium hydroxide 100 g., distilled water 250 ml. Dissolve, cool, and keep iced while adding *guttatim* bromine 25 ml. Mix and dissolve. This solution is used to estimate the amount of urea in a given quantity of urine. On adding the solution, nitrogen is evolved from the urea and is measured at atmospheric pressure. It is better to keep the bromine separate; 1 ml. of bromine should be added to 11 ml. of the solution as required.

A 100 ml. graduated tube is connected at the lower end by rubber tubing with a levelling tube, and at the upper end with a small bottle. The bottle is closed by a rubber cork having two holes with glass tubing through the holes, one being connected by rubber tubing to the upper end of the graduated tube, the other closed by a short piece of rubber tubing and screw clip. The bottle contains a small test-tube which will just lie obliquely. Put 25 ml. of hypobromite solution in the bottle and 4 ml. of urine into the test-tube. Insert cork and, with screw clip open, adjust the levelling tube until water in the graduated tube is at the 0 mark; close clip. Tilt bottle until all the contents of the test-tube are spilled into the hypobromite. When the reaction has finished, adjust levels and read off number of millilitres of gas evolved. Each millilitre of nitrogen $\times 0.0625$ = the percentage of urea present.

Xanthidrol has been applied to the quantitative determination of urea in urine with which it forms dioxanthylurea, $[O(C_6H_4)_2CH \cdot NH]_2CO$. Other constituents of urine do not interfere.

To 5 ml. of urine add 3.5 ml. of glacial acetic acid and 1 ml. of 10% solution of the reagent in methyl alcohol, followed by four further additions of 1 ml. at 10-minute intervals. After one hour, collect the precipitate on a sintered glass filter, wash twice with acetic acid (66%), then with alcohol and finally remove alcohol with water. Grind the precipitate with small portions of a cold mixture of equal volumes of N/1 potassium dichromate and concentrated sulphuric acid, the volume of the mixture being gradually increased until 50 ml. in all has been added; then boil for 5 minutes. Cool, dilute to 250 ml., and titrate the excess dichromate in an aliquot portion by means of iodide and sodium thiosulphate. One molecule of dioxanthylurea requires 58 atoms of oxygen, and 1 ml. of N/1 potassium dichromate is equivalent to 0.517 mg. of urea.—per A. D. Mitchell, Lecture to Institute of Chemistry, Oct. 19, 1934.

Urease Method of Estimating Urea. Mix 25 ml. of the urine with a pinch of powdered soya-bean flour (2 to 3 g.). Allow to stand overnight covered with a small layer of xylol or benzene. Render the liquid alkaline with strong sodium carbonate solution and distil into standard hydrochloric or sulphuric acid by Kjeldahl's procedure. Urease only attacks urea, 1 molecule of urea producing 1 molecule of ammonium carbonate, $(NH_4)_2CO_3$, and there may be present a small amount of ammonium salts in addition to free ammonia. For accurate work these must be estimated separately.

Urease Preparation. Cover 200 g. of finely powdered soya bean with 1000 ml. of water and keep 6 hours. Treat the filtrate with 96% alcohol (400 ml.), as long as precipitate forms. Collect and dry slowly and add lactose *q.s.* to 100 g. Keep the product dry.

Urea Nitrogen Determination by Direct Nesslerisation. Urease is used as above for hydrolysis of the urea, either in the form of the enzyme or as soya

bean meal. It decomposes urea quantitatively and does not affect other constituents of urine.

Place 1 ml. of the urine in a 100 ml. graduated flask. Add 0.1 to 0.25 g. of soya bean meal in the form of a 1% suspension. Allow to stand for one hour at room temperature, or 15 minutes at about 50°. Add 25 ml. of water and 1 ml. of *m*-phosphoric acid solution (25%) and mix, then add 1 g. of pure blood charcoal, shake, make up to volume, mix, and filter.

(The soya bean meal suspension is made thus: Rub 5 g. with water 15 ml. to a smooth paste. Add more water *q.s.* to about 400 ml. Add 100 ml. of alcohol. 10 to 15 ml. of this are used. It keeps good for about 2 days.)

Place 5 to 20 ml. of the filtrate in a 100 ml. graduated flask. (The amount taken should contain 0.7 to 1.3 mg. of ammonia nitrogen.) Dilute to 60 or 70 ml. Nesslerise with the modified Nessler reagent, (*v. infra*), and compare with standard 1 mg. of ammonia nesslerised in another 100 ml. flask).

The determination of minute quantities of urea by hydrolysis with acid at 150° followed by nesslerisation.—J. T. Wear and A. N. Richards, *J. biol. Chem.*, 1925, 66, 275.

The **total nitrogen** of urine varies considerably with the protein intake but the average 24 hours excretion is about 16 g. Of this the urea nitrogen is 14 g., ammonia nitrogen 0.5 g., creatinine nitrogen 1.5 g., uric acid nitrogen 0.25 g., with a little undetermined nitrogen. The fluctuations in amount are nearly always due to variations in the urea excretion which accounts for about 90% of the total nitrogen. Nitrogen is best estimated by some form of Kjeldahl's method. 5 ml. of urine is heated in a Kjeldahl flask with 10 ml. of concentrated sulphuric acid, a few crystals of potassium sulphate and 0.5 ml. of saturated copper sulphate solution until all organic matter is destroyed. The solution is diluted with water and after cautious addition of an excess of 40% sodium hydroxide the ammonia formed is distilled into sulphuric acid. The amount of N/5 sulphuric acid neutralised by the ammonia is found by titration with N/10 sodium hydroxide.

Folin and Denis' Method of Estimating Total Nitrogen. Modified Nessler Reagent. Folin and Denis criticise the composition of Nessler's reagent as being excessively alkaline and containing too little potassium iodide. They dissolve potassium iodide 75 g. in warm water 50 ml., add mercuric iodide 100 g. and stir. Dilute with water 400 or 500 ml., filter and make up to 1 litre. To 300 ml. of this double iodide solution add 200 ml. of 10% sodium hydroxide, 500 ml. of water and mix. This final solution contains 2% of sodium hydroxide, which is preferable for Nesslerising digestion mixtures of samples of urine.

15 ml. of this reagent added quickly to the digestion mixture will yield *clear* mixtures with as large amounts of ammonia as are met with in the method (0.7 to 1.6 mg. ammonia nitrogen).

The **ammonia nitrogen** rises to relatively high levels in all conditions of acidosis. The usual ratio of ammonia nitrogen to urea nitrogen is 1 to 20. In severe acidosis it may be 1 to 2.

Ammonia nitrogen may be rapidly estimated in urine by the formaldehyde method. 25 ml. of urine to which have been added a few crystals of neutral potassium oxalate are titrated to the neutral point of phenolphthalein with N/10 sodium hydroxide. To this is added 5 ml. of 40% formaldehyde previously neutralised to phenolphthalein. Formaldehyde reacts with neutral ammonium salts liberating an equivalent amount of acid. $4\text{NH}_4\text{Cl} + 6\text{H}\cdot\text{CHO} = \text{N}_2(\text{CH}_3)_4 + 4\text{HCl} + 6\text{H}_2\text{O}$. The acid produced is titrated with N/10 sodium hydroxide. 1 ml. of N/10 sodium hydroxide is equivalent to 0.0014 g. of ammonia nitrogen.

This method also estimates any amino-acids that may be present, but these are negligible except in rare circumstances.

The ammonia in urine may be estimated by distillation and Nesslerisation of the distillate or by aid of volumetric acid, as above.

Uric Acid

The average content in the urine is 0.05 to 0.06%. This is derived from endogenous and exogenous sources. The exogenous uric acid fluctuates with the amount of nucleoprotein in the diet. Estimations of uric acid in the urine are only of value where diet is known and uric acid excretion over a period is being estimated. In gout there is a deficient excretion of uric acid.

When pure, uric acid occurs as white crystals, very slightly soluble in water, insoluble in alcohol and ether.

Murexide Reaction. On heating to dryness on a water-bath with a little nitric acid or potassium chlorate and hydrochloric acid in a white dish, cooling, and adding a little ammonia solution a red colour is produced in the presence of uric acid.

Hopkins' Method. To 100 ml. of sample add about 30 g. of ammonium chloride in powder, dissolve as completely as possible, or a small quantity may remain undissolved, add a little ammonia to neutralise and allow to stand for 10 minutes. Filter off the precipitated acid ammonium urate, wash with saturated ammonium sulphate solution and rinse off the precipitate from the filter with water to 100 ml. Add 20 ml. concentrated sulphuric acid to raise temperature of the liquid to about 60°, or, if necessary, warm to that temperature. Titrate with N/20 potassium permanganate (1.58 g. in 1 litre), taking as end-reaction the point at which the permanganate ceases to be instantly decolorised. Each ml. of the permanganate solution = 0.00375 g. uric acid.

Gowland-Hopkins' Method is the same as the above until the acid ammonium urate has been washed with ammonium sulphate solution, then proceed as follows:—Wash off the precipitate into a small beaker with a jet of hot water, add a little hydrochloric acid, and heat just to boiling. Allow to stand two hours in the cold. Collect the separated uric acid, measuring the filtrate at the same time, for which an allowance of 1 mg. must be added on to the final result for every 15 ml.; it need not exceed 20 to 30 ml. Wash the uric acid crystals with a little distilled water, rinse off the filter with hot water, warm with sodium carbonate till dissolved and make up with water to 100 ml. Add 20 ml. of sulphuric acid and titrate with permanganate as above, adding it slowly towards the end of the reaction, the finish being the first appearance of a pink colour which is permanent for an appreciable interval. Previously the disappearance of the colour is instantaneous.

Phosphotungstic Acid Test. (A rapid approximation.) Mix about 10 ml. of urine with 3 ml. of solution of potash, add 20 drops of a 20% solution of phosphotungstic acid. Uric acid causes a blue colour which varies in depth with the proportion present. The method is not applicable for anything approaching an accurate colorimetric estimation since the colour fades rapidly. Use a standard for comparison of 1 in 50,000 uric acid.

The test can also be conducted by heating the urine with solution of potash and a 5% solution of phosphotungstic acid, which gives a lilac colour. The intensity can be compared with that given by a standard solution of uric acid 1 in 1000.

The following modification of Folin's method for the colorimetric determination of uric acid avoids difficulties due to the turbidity of the final solutions:—1 ml. or more of urine is mixed with 3 ml. of water and 3 ml. Folin's acid silver lactate solution, allowed to stand in the dark for a few minutes, and centrifuged. The precipitate is dissolved in 5 ml. of Folin's cyanide and urea reagent (see below), and the solution transferred quantitatively to a 100 ml. flask by means of 2 portions of 10 ml. each of 10% sodium carbonate solution and 5 ml. of water. 5 ml. of uric acid reagent is added, the contents mixed and then after 5 minutes diluted to 100 ml., and the colour compared with a standard prepared by diluting to 100 ml. 5 ml. of standard uric acid solution containing 0.1 mg. of uric acid per ml., 5 ml. of cyanide and urea reagent, 20 ml. of 10% sodium carbonate solution, and 5 ml. of the uric acid reagent.—H. B. Salt, *Biochem. J.*, 1931, 1720.

The acid silver lactate solution is prepared by dissolving 100 g. of silver lactate in 700 ml. of water containing 100 ml. of lactic acid (85%) partly neutralised by 100 ml. of 10% sodium hydroxide.—O. Folin, *J. biol. Chem.*, 1922, 54, 153.

Cyanide-Urea Reagent. Add 50 g. of sodium cyanide to 700 ml. of water and stir until dissolved. Add 300 g. of pure urea and stir until practically complete solution is obtained. Transfer to a 2 litre flask, add 5 to 6 g. of pure calcium oxide and shake for 5 minutes. Filter and store in tightly stoppered bottles.—O. Folin, *J. biol. Chem.*, 1930, 86, 179.

Renal Function Tests

The tests are of chief value in certain surgical conditions, in the albuminurias of pregnancy and in the diagnosis and prognosis of medical cases of kidney disease. In surgical cases of obstruction the blood urea has been found to be the most useful test of kidney function. A blood urea content above 60 mg. per 100 ml. contra-indicates prostatectomy in one stage. In other types of surgical cases the dye excretion tests are most satisfactory and are usually performed by the surgeon himself.

In cases of albuminuria in pregnancy with clinical symptoms such as headache, vomiting and œdema, as in nephritis toxæmia (de Wesselow) there is marked nitrogen retention, with a blood urea above 40 mg. per 100 ml. and a high blood pressure. In threatened eclampsia there is albuminuria, diminished urea output and frequently an increased blood urea, but the imminence of eclampsia cannot be judged from the amount of nitrogen retention.

In medical cases of kidney disease, renal function tests are often useful but it must be borne in mind that obstruction to urinary outflow produces much greater nitrogen retention than severe kidney disease. Blood analysis is particularly useful in checking the effects of diet in cases of nephritis with nitrogen retention. Similarly in parenchymatous nephritis the value of treatment can be assessed by the reduction in blood cholesterol. Whatever tests are performed their value must always be weighed in the light of information gained from general clinical examination, the blood pressure and condition of the arteries.

Of the numerous tests devised for estimating the functional activity of the kidneys the following have proved the most reliable in practice. (*For details of blood urea estimations, see Blood Analysis, p. 697.*)

(1) **Systematic examination of the urine** (including the record of the total quantity passed each day, the specific gravity, the presence of albumin, blood, and pus, and the presence of casts). If the patient is on a standard diet and accurate daily quantitative tests can be made, useful information can be obtained from quantitative tests of urinary constituents, particularly of the urea and chlorides in the urine, but this method has only a limited applicability in practice. The following table (from *Recent Advances in Medicine*, by Beaumont and Dodds 4th Edn., p. 23), gives figures obtained at the Middlesex Hospital.

Table showing analyses of twenty-four hours' urine of typical cases of renal inefficiency.

| Case. | Vol. ml. | Albumin per 1000 | Urea g. | Uric Acid g. | Creatinine g. | Total N. g. | Chlorides g. |
|--------------------------------|----------|------------------|---------|--------------|---------------|-------------|--------------|
| Normal men .. | 1500 | — | 30 | 0.6 to 1.2 | 1 to 1.25 | 14 to 16 | 10 to 15 |
| Acute nephritis | 300 | 20 | 7 | 0.2 | 0.8 | 6 | 1.7 |
| Chronic interstitial nephritis | 3000 | 0.5 | 15 | 0.6 | 0.9 | 8 | 14.8 |
| Large white kidney .. | 1000 | 10 | 14 | 0.72 | 0.85 | 7 | 1.7 |
| Small white kidney .. | 1800 | 12 | 12 | 0.84 | 0.79 | 6.4 | 0.9 |

Water Concentration Test. Ordinary meals are allowed but no fluids drunk for 24 hours. The urine of the last 12 hours is collected and the specific gravity measured. A healthy adult under these circumstances (Addis and Shevky) excretes urine with a sp. gr. of between 1.027 and 1.032. The lower the sp. gr. of the urine the greater the damage to the kidney. When the sp. gr. is 1.026 the chance of the kidney being healthy is 1 in 47, and when it is 1.022 the chance of a healthy kidney is 1 in 15,000.

Water Dilution Test. 1200 ml. (43 oz.) of water are given to a patient without any breakfast and an additional 900 ml. (32 oz.) in the next 5 hours. The urine passed during the last five hours should have a sp. gr. of 1.003 and if it is greater than this the kidney is definitely deficient.—G. Graham, *Med. Pr.* ii/1936, 504.

(2) **Elimination Tests.** The *Urea Concentration Test* is the most popular. The principle is to give a large dose of urea (15 g.) *per os* and observe how rapidly the kidney removes the excess. The test is best carried out first thing in the morning.

Technique. The patient is allowed no food or drink after 10 p.m. the previous night. At 5.58 a.m. the bladder is emptied completely and this specimen of urine marked "0." At 6 a.m. he takes the following mixture: urea 15 g., tincture of orange 1 ml. and water 100 ml. At 7 a.m. he empties his bladder completely, this specimen being marked "1." At 8 and 9 a.m. he passes urine again, these specimens being marked "2" and "3" respectively. The total quantity of urine passed at 7, 8 and 9 a.m. must be measured. This should not exceed 120 ml. in No. "1" and 100 ml. in Nos. "2" and "3." If more than this is passed it indicates that the urea has a diuretic action and a low urea concentration may not necessarily mean a poor renal function.

Normally the concentration of urea in one or other specimen is at least 2.5 or 3%. In renal inadequacy it is below 2%.

The chief limitations of the test are that in the hydræmic type of nephritis with chloride retention it may give normal results even when the patient is very ill, because in this disease there is not necessarily any nitrogen retention. In cases of enlarged prostate with residual urine the first two specimens may be

influenced by dilution with this residue but by the third hour the real concentration should be apparent. Sometimes it is useful to collect a specimen at 4 hours.

Phenolsulphonephthalein (Phenol red) Test. Give 300 to 400 ml. of water half an hour prior to the test. Empty the bladder with a catheter and give intramuscularly, or preferably intravenously, in the upper arm 6 mg. of phenolsulphonephthalein neutralised with sodium hydroxide in 1 ml. of water. The patient is then catheterised and the urine allowed to drop into a beaker containing 2 drops of 25% sodium hydroxide. Normally a red colour, indicating excretion of the dye, begins to appear in the urine within 5 to 10 minutes. The colour reaches its maximum intensity within 15 to 20 minutes and the dye is all excreted within 4 hours. Samples of urine are collected at the end of one and two hours, and to each sample 25% sodium hydroxide is added sufficient to develop the maximum red colour and the mixture diluted to 1000 ml. with distilled water. As a standard 3 mg. of phenol red is added to some of the urine obtained before the injection, the volume then being made up to that passed during the second hour, 25% sodium hydroxide added to develop the maximum colour and the mixture diluted to 1000 ml. The samples and the standard are then filtered, the filtrates compared in a colorimeter, and the percentage of dye excreted in the first and second hours calculated. Figures below 40% for the first hour or 60% for the first and second hours combined (70% in children) suggest renal inefficiency.

In severe acute nephritis the permeability is markedly decreased, also in chronic interstitial nephritis. The delayed appearance and especially the diminished excretion in the 2-hour period are more accurate indications of functional derangement than an estimation of total solids or nitrogen.

In the stools of 9 out of 26 patients, after intravenous injection of 6 mg., 1 to 8% of the amount injected was recovered, showing that the dye may be eliminated, re-absorbed, and transformed in the digestive tract. The duodenal tube, immediately after the injection, showed presence of 1 to 3% of the dye in the bile of three normals, while it was absent in the bile of five persons with liver disease.—*J. Amer. med. Ass.*, ii/1925, 309.

By tests on normals in which the urine was collected at 15-minute intervals for 2 hours, the curve of dye elimination by the kidneys was shown to be 40% during the first 15-minute period, 17% during the second, 8% during the third, and 4% during the fourth, gradually decreasing to 0.5% during the eighth. A series of cases of known renal insufficiency showed abnormalities in the curve, the presence of an abnormal curve indicating impending renal failure, while the other tests were negative.—*J. Amer. med. Ass.*, ii/1925, 469.

The 1- and 2-hour specimens are no longer obtained since the curve of dye elimination with a high initial output (normal minimum 25%) is the significant feature of 'phthalein' excretion. One third of 43 patients with Bright's disease had a total dye output of 55% or more by the old method of hourly collections, yet in each there was a delay in the dye excretion shown only by the fractional method. This delay, reflected chiefly in the 15-minute output, indicated an impaired kidney function.—*E. M. Chapman, New Engl. J. Med.*, i/1936, 16.

Indigo Carmine Test. 10 ml. of a 0.4% solution is injected intravenously. Cystoscopic examination of the urethral openings and the urine gives, by depth of colour, indication of renal functional power. The cystoscope should be introduced immediately after the injection and the urethric orifices carefully watched, when the ejection should appear as a forcible dark blue jet. Parenchymatous or interstitial nephritis is suggested by delay in excretion from both ureters. Marked delay on one side indicates disease; thus, if 8 to 12 minutes after injection, suggests chronic pyelitis. If 12 to 18 minutes elapse before elimination, partial urethral obstruction or moderate impairment of renal function is indicated. 20 minutes' delay indicates almost complete obstruction, or serious disease of the kidney or ureter.

In normal kidneys the colour appears within 5 to 20 minutes, the maximum colour should appear in 45 minutes, and total excretion should be complete in 14 hours.

Methylene Blue Test. 1 ml. of 1 in 20 solution is injected into the gluteus maximus and the urine is turned pale green in half an hour, the colour increasing up to the fourth hour. The method is sufficient to compare the work of the two kidneys, but the indigo carmine method is better.

Phloridzin Test. This consists in injecting 5 to 10 mg. of phloridzin subcutaneously in 20 to 30 minims of water. Glucose should normally appear in the urine in half an hour. This test is also delicate for determining which kidney is diseased.

The technique of Caspar's method consists in the subcutaneous injection into the buttock of 1 ml. of 1% phloridzin solution and observation as to (a) excretion of sugar by a healthy kidney or (b) non-excretion, or more slowly and to less extent (diseased).

Creatinine Test. In normal persons and those with no real lesions the intravenous injection of 0.5 g. of creatinine is followed by increased excretion. In chronic nephritis the increase is either *nil* or under 50%. It should serve as a useful confirmation of other methods.—R. H. Major, per *Prescriber*, 1923, 160.

Van Slyke's Blood Urea Clearance Test.

Definition of Augmentation Limit. When the urinary volume is relatively large the amount of urea excreted per minute is directly proportional to the blood urea content. When the urinary volume is small this relation no longer holds. Van Slyke *et al.* called the urinary volume above which this relation does hold the "*augmentation limit*" (augmentation of urinary volume beyond this limit does not increase the rate of urea excretion). The usual augmentation limit in adults is about 2 ml. of urine per minute. When the urinary volume is 2 ml. or more per minute, calculate the maximum blood urea clearance. When the urinary volume is below 2 ml. per minute, calculate the standard clearance.

Maximum Blood Urea Clearance. Above the augmentation limit, increase of urinary volume does not increase the amount of urea excreted in urine per minute; i.e., the average amount of urea excreted per minute remains constant. Therefore, on the basis of the idea that the blood is *completely* cleared of urea as it passes through the kidneys (which, of course, it is not), the number of millilitres of blood cleared of urea per minute remains constant. Let this maximum clearance be denoted by C_m .

Suppose the volume of urine excreted per minute = V ml.,
and the concentration of urea in this urine = U mg. per ml.
Therefore, urea excreted in urine per minute = $U \times V$ mg. Since $U \times V$ mg. of urea are excreted in urine per minute, therefore the same amount ($U \times V$ mg.) must be removed from the blood in one minute. Suppose the blood contains B mg. of urea per 1 ml.

Therefore 1 mg. of urea is contained in $\frac{1}{B}$ ml. of blood,

therefore $U \times V$ mg. of urea is contained in $\frac{U \times V}{B}$ ml. of blood,

i.e., $\frac{U \times V}{B}$ ml. of blood are completely cleared of urea per minute,

or $C_m = \frac{U \times V}{B}$ ml. of blood per minute.

Provided that U and B are expressed in the same terms, it does not matter whether mg. per 1 ml. or mg. per 100 ml. or g. per cent. or g. per litre is used. The urinary volume V , however, must be expressed in millilitres of urine per minute.

Example.

$$V = 150 \text{ ml. per hour} = 2.5 \text{ ml. per minute.}$$

$$U = 1\% = 1000 \text{ mg./100 ml.} = 10 \text{ mg. per 1 ml.}$$

$$B = 50 \text{ mg./100 ml.} = 0.5 \text{ mg. per 1 ml.}$$

$$C_m = \frac{UV}{B} = \frac{10 \times 2.5}{0.5} = 50 \text{ ml. of blood per minute}$$

or since average normal = 75 ml. of blood per minute,

therefore $C_m = \frac{50}{75} \times 100 = 67\%$ of average normal.

Standard Blood Urea Clearance. Below the augmentation limit, the number of millilitres of blood cleared of urea per minute is not constant, but varies, on the average, with the square root of the urinary volume. It is, therefore, necessary to fix the urinary volume at a definite standard (which is not practicable), or to calculate by means of the square root rule from the observed urea

excretion what would be the urea excretion which would accompany such a standard urine volume.

Suppose C is the observed blood urea clearance when the urinary volume is V ml. per minute. Let C_s be the standard blood urea clearance with a corresponding standard urinary volume of V_s ml. per minute.

$$\text{Then } C_s : C :: \sqrt{V_s} : \sqrt{V}$$

$$\text{or } C_s = \frac{C \sqrt{V_s}}{\sqrt{V}}$$

As standard urinary volume (V_s), Van Slyke *et al.* adopt 1 ml. per minute = 1440 ml. per 24 hours).

$$\text{Therefore } C_s = \frac{C \times 1}{\sqrt{V}}$$

But C , the observed clearance, as we have seen for C_m , the maximum clearance, is equal to $\frac{U \times V}{B}$

Substituting for C

$$C_s = \frac{U \times V}{B} \times \frac{1}{\sqrt{V}}$$

$$\text{i.e., } C_s = \frac{U \times \sqrt{V}}{B} \text{ ml. of blood per minute.}$$

In health the average values of C_m and C_s have been found to be 75 ml. and 54 ml. respectively.

Results can, therefore, be expressed as percentages of average normal, instead of as so many millilitres of blood per minute. Thus comparisons can be made between C_m of one patient and C_s of another patient.—*J. clin. Invest.*, 1928, 6, 47; *Quantitative Clinical Chemistry*, Peters and Van Slyke, Vol. I, p. 345, and Vol. II, pp. 564, 935.

In carrying out the test, the patient, who is on an ordinary diet but resting in bed, empties his bladder at the commencement. After an exactly measured period (e.g., 1 hour) the bladder is again emptied and the urea excreted is determined. After a further similar period, the determination is repeated as a control. The blood urea is determined at about the middle of each period. A glass of water may be taken at the commencement of the test and at the end of the first period.

A defect in renal function might be revealed by this test when blood urea was still within normal limits. The influence of a minor surgical operation on renal function showed that on the day following operation the urea clearance value fell from 76 to 52% but returned to original level next day. Really nothing to worry about until figure shown by the test was 40% or less.—C. Dukes, *Brit. med. J.*, i/1934, 209.

From a surgical point of view the most valuable test available, especially in prostatic cases where a figure of 60% might be taken as the border line to indicate whether a one-stage or two-stage operation should be performed.—E. W. Riches, *ibid.*, 209.

Modifications of Van Slyke's Test.

POWWEATHER'S METHOD. This consists in giving 15 g. of urea in 4 oz. of orange juice (the urea should not be given if the blood urea is over 60 mg.) and the test is then carried out in the usual way. The results of the analyses of the first and second hours agree more closely than with the Van Slyke urea clearance test.—G. Graham, *Med. Pr.*, ii/1936, 505.

ADDIS'S METHOD. A dose of 30 g. of urea is given (provided the blood urea is not over 60 mg.) together with 1000 ml. (35 oz.) of water. This amount of water is given every hour for the next 5 hours and the urine of the fourth, fifth and sixth hours collected. The blood urea is estimated during the course of each of the last 3 hours. The method ensures that the blood urea is between 60 and 90 mg., so that plenty of urea is excreted and that more than 120 ml. of urine

is passed each hour. The "maximum" clearance is obtained by this method and the results of each hour agree closely with each other. The test is more complicated and unpleasant than the simpler urea clearance test.—G. Graham, *Med. Pr.*, ii/1936, 507.

Other Substances in Urine

Bromides. The examination of urine for the presence of bromides is of value in the diagnosis of drug rash due to bromide intoxication. Bromides are excreted as such.

A simple test for the presence of bromides consists in shaking up the urine with charcoal and filtering. To 5 ml. of the filtrate add 1 ml. of 20% trichloroacetic acid and 1 ml. of 0.1% gold chloride. If positive, a brown colour appears. Alternatively to 10 ml. of urine add 2 ml. of 10% *v/v* sulphuric acid and a few drops of potassium permanganate solution until the mixture assumes a permanent pink colour. On heating, the mixture turns a brown colour and gives the characteristic smell of bromine.

Fluorescein Test for Bromine in Body Fluids. Soak strips of filter paper in a saturated solution of fluorescein in 60% acetic acid, and allow to dry. Add to body fluid in a test tube a few crystals of potassium permanganate. Agitate and add a few drops of concentrated sulphuric acid. Moisten fluorescein paper with 2% acetic acid and hold at mouth of test-tube. Presence of bromine indicated by rapid change of paper from yellow to bright pink. Presence of chlorine and iodine does not interfere with test. Found positive in urine voided 15 minutes after oral administration of 10 grains of sodium bromide. The fluorescein papers will keep.—G. H. Belote, *J. Amer. med. Ass.*, i/1927, 1697.

Determination of Minute Quantities of Bromide in Presence of Chloride. The following method is suggested for quantities of less than 1 mg. 20 ml. of the solution is treated in a conical flask with 2 ml. of approximately N/1 calcium hypochlorite, 4 drops of 2N hydrochloric acid and enough calcium carbonate to render the liquid permanently milky. The mixture is boiled gently for 5 minutes and then treated, drop by drop, with 1 ml. of 20% sodium formate to decompose the excess of hypochlorite. The liquid is boiled for a further 5 minutes, cooled, and acidified with 2-3 ml. of hydrochloric acid; a few crystals of potassium iodide and 1 drop of 10% ammonium molybdate solution (as catalyst) are then added and the solution titrated with N/500 sodium thiosulphate from a microburette, starch solution being added near the end of the titration. A blank test should also be made since the hypochlorite may contain bromine. By increasing the quantities the process can be used for larger quantities (e.g., 50 mg. of bromine).—H. Doering, *Z. anal. Chem.*, 1937, 108, 255.

A method for the estimation of bromides in urine in the presence of large amounts of chlorides.—G. H. W. Lucas, *J. Pharmacol.*, 1928, 223.

Cushny found that after a single dose of 30 grains of bromide the urine may contain traces of the drug for 2 months, only about 10% being eliminated in the first 24 hours.—J. H. Hannan, *Practitioner* ii/1927, 262.

Calcium. The average daily excretion of calcium (calculated as CaO) in the urine is from 0.1 to 0.4 g., the excretion varying according to the amount of calcium in the diet. The greater proportion of the calcium ingested is normally eliminated in the fæces. Excessive urinary calcium excretion occurs in osteomalacia, while in rickets the excretion may be very low. The Sulkowitch test is of value for the detection of excessive excretion in patients suspected of hyperparathyroidism, and for the control of treatment of tetany with dihydrotachysterol.

Shohl and Pedley's Method. In this method for the determination of calcium (*J. biol. Chem.*, 1922, 50, 537) the urine is oxidised with ammonium persulphate. Calcium is precipitated as oxalate and titrated with potassium permanganate.

Sulkowitch Test. 5 ml. of a buffered oxalate solution are added to an equal amount of urine which is acid to litmus, the test-tube is inverted and shaken and the turbidity caused by the precipitation of calcium oxalate is observed after 2 minutes. An immediate dense cloud indicates a high concentration of calcium which demands further investigation and the absence of any precipitate almost always rules out excessive parathyroid activity. When the test is done the diet must be free from milk and acidifying agents which may cause temporary increases in calcium excretion.—G. C. Linder and J. M. Latsky, *Lancet*, i/1942, 105.

Lead. Amounts up to 0.1 mg. of lead per litre are commonly found in the urine of healthy individuals, but amounts in excess of 0.2 mg. per litre are indicative of lead poisoning, though the diagnosis continues to rest largely on clinical evidence. More of the metal is excreted in the faeces than the urine and both excretions should be analysed.

Determination of Lead in Urine with Diphenylthiocarbazone. The diphenylthiocarbazone reagent is prepared as follows. A 0.05% solution of dithizone in chloroform is shaken with successive quantities of 10% ammonia solution until no longer of a green colour. The mixed ammoniacal extracts are acidified with dilute sulphuric acid and the precipitate redissolved in pure chloroform to give an approximately 0.05% solution which is stored in dark coloured bottles and diluted with an equal volume of chloroform as required for use. 500 ml. of urine is treated with 10 ml. of strong ammonia solution, allowed to stand overnight and the precipitated phosphate filtered off. The sides of the beaker are washed down with a small amount of 2% ammonia solution and the washings passed through the filter, which is then dried by gentle suction and ashed in a silica basin for 1 hour at about 500° in a muffle, the heating being repeated for 30 minutes after the addition of a few drops of nitric acid. The cooled white residue is boiled with 20 ml. of dilute hydrochloric acid (10%) and filtered, the filter washed with hot water 5 or 6 times, the filtrate and washings allowed to cool and 10 ml. of 60% ammonium citrate solution added. The liquid is made just alkaline to litmus with 10% ammonia solution. The clear alkaline solution is shaken vigorously in a 300 ml. separator with 5 ml. of dilute dithizone solution. In the presence of lead the chloroform is coloured pink or violet. The solvent is run off and the aqueous liquid extracted with successive quantities of 5 ml. of dilute dithizone solution until the separated chloroform is no longer coloured pink after shaking with 5 ml. of 1% potassium cyanide solution mixed with 5 ml. of 2% ammonia solution and 10 ml. of water. Free dithizone is removed by this washing which is repeated several times on the mixed chloroformic extracts, any chloroform removed being collected by storing the mixed cyanide washings until it has separated and removing it in a separator and returning to the main bulk. The chloroform extract is washed with water and shaken with 15 ml. of N/10 hydrochloric acid which decomposes the pink lead dithizone compound. The green chloroform solution is transferred to a 50 ml. flask, the acid layer being washed with more chloroform, and made up to the mark. It is then compared colorimetrically with a standard lead solution similarly treated.—F. Morton, *Analyst*, 1936, 465.

Determination in Tissues and Excreta. For lead in urine, 500 ml. is evaporated, ignited in a silica dish, 5 ml. of nitric acid added and the whole again ignited. The ash is dissolved in 100 ml. of water containing 5 ml. of hydrochloric acid, 100 ml. of a 20% solution of sodium citrate is added, and the mixture made alkaline to litmus with strong solution of ammonia. 10 ml. of a 2% solution of sodium diethyldithiocarbamate is added and the liquid is extracted with 25 ml. of ether, the ether extract is separated, washed twice with 25 ml. of water, and the combined aqueous liquids are again extracted with ether. The combined ethereal extracts are evaporated in a Kjeldahl flask, and heated with 1 ml. of sulphuric acid and 1 ml. of perchloric acid to destroy organic matter, fumes being removed by a water-pump. After digestion, 10 ml. of water, 1 ml. of glacial acetic acid, and 5 ml. of strong solution of ammonia are added in this order, and the volume is adjusted to 25 ml. with water. 5 to 10 ml. of this solution is transferred to a 50-ml. volumetric flask and 6 drops of a 5% sulphurous acid solution are added for each 5 ml. of solution taken; 5 ml. of a 1% solution of potassium cyanide is then

added, followed by 10 ml. of carbon tetrachloride and 0.5 ml. of 0.1% solution of diphenylthiocarbazon, and, after vigorous shaking, the aqueous solution is removed by means of a pipette. The organic liquid is washed four to six times with 10-ml. quantities of a 1% solution of potassium cyanide to remove excess of diphenylthiocarbazon and, after washing with water, the pink organic extract is compared in a colorimeter with standard solutions prepared at the same time in a precisely similar manner using 1 to 2 ml. of a standard lead solution equivalent to 0.01 to 0.02 mg. of lead. The aqueous layer, after extraction with carbon tetrachloride should be coloured brown, indicating the presence of excess of reagent. For soft tissues, 100 g. of the finely chopped material is mixed with 100 ml. of a 10% solution of sodium phosphate, evaporated, ignited, and treated as for urine. For bone or faeces 5 to 10 g. is ignited directly. For blood, about 20 ml. is withdrawn from the patient, rapidly pipetted into 100 ml. of 10% solution of sodium phosphate, evaporated to dryness and ashed with 1 ml. of nitric acid. The determination is continued as for urine, using only one-fifth the volume of reagents specified, except that the extraction with ether and carbon tetrachloride is performed with the quantities previously specified. In persons not exposed to lead poisoning, the average amounts of lead found in mg. per kg. were:—liver, 1.73; kidney, 1.35; spleen, 1.68; brain, 0.50; rib, 8.55; vertebra, 7.1; total lead in right lung, 0.50. In human fetuses the average concentration of lead is between one-half and one-third that found in normal adults.—S. L. Tompsett and A. B. Anderson, *Biochem. J.*, 1935, 1851.

Sex Hormones. Œstrogens and androgens are present in the urine of both sexes, and although there is some evidence that the former are in excess in female and the latter in male urine, there is at present no method of assay of these substances that will identify the urine as being derived from male or female. The substances may be assayed by biological methods, using the comb growth of capons for androgenic activity and ovariectomised mice for Œstrogens. The substances present in the urine are not the original hormones as found in the tissues, but degradation products having only a portion of the activity of the parent substances. That such substances are not entirely of gonadal origin is shown by their presence in the urine of eunuchs, and there is no doubt that the suprarenal cortex supplies some such material. A colorimetric method for the estimation of these 17-keto-steroids has been fully described by Callow *et al.*

Russell Fraser *et al.* showed that the normal range of 24-hour excretion of 17-keto-steroids in males between the ages of 20 and 40 was 8.1–22.6 mg., with an average of 13.8 mg., and in a similar series of females the figures were 5.1–14.2 mg., with an average of 9 mg.

Callow's Method for Estimation of 17-keto-steroids. The colorimetric method bears a fairly close relationship to the androgenic activity as estimated by comb growth in the biological test. 17-keto-steroid excretion is lowered in malnutrition, gastro-intestinal disease, anæmia and infections. Diseases of the liver show a fairly marked decrease. Results of assays on endocrine cases indicate that of the urinary 17-keto-steroids those derived from the male gonads represent about 5 mg. and from the adrenal cortex about 9 mg. Fraser *et al.* record the following figures calculated from their extensive series of assays:—

| | | | | | |
|---------------------------------|----|----|----|----|--------|
| Normal male | .. | .. | .. | .. | 14 mg. |
| Normal female | .. | .. | .. | .. | 9 mg. |
| Female with Addison's disease | .. | .. | .. | .. | 0 |
| Male with Addison's disease | .. | .. | .. | .. | 5 mg. |
| Panhypopituitarism (either sex) | .. | .. | .. | .. | 0 |
| Gonadectomised male | .. | .. | .. | .. | 9 mg. |
| Gonadectomised female | .. | .. | .. | .. | 9 mg. |

A patient with adrenal tumours showed a very high 17-keto-steroid excretion. Excretion of androgens by eunuchs. The isolation of 17-keto-steroids from the urine.—N. H. Callow and R. R. Callow, 1940, *Biochem. J.*, 34, 276.

The differential diagnosis of forms of basophilism by estimation of the urinary androgens.—Crooke and Callow, *Quart. J. Med.*, 1939, 32, 233.

17-keto-steroid, androgen and oestrogen excretion in the urine of cases of gonadal or adrenal cortical deficiency.—Callow and Emmens, *J. Endocrinology*, 1940, 88.

Methods of extracting compounds related to the steroid hormones from human urine.—Callow *et al.*, *J. Endocrinology*, 1939, 76.

Colorimetric assay of 17-keto-steroids in urine. A survey of the use of this test in endocrine investigation, diagnosis and therapy.—R. W. Fraser *et al.*, *J. Endocrinology*, 1941, 234.

Pregnanediol Excretion in the Diagnosis of Pregnancy Disorders. Pregnanediol, which is similar in chemical structure to progesterone, is found in the urine only when a corpus luteum or a placenta is actively functioning in the body. It is thus present only during the luteal phase of normal menstruation and during pregnancy and it is probably an excretion product of progesterone metabolism. It is excreted in the urine as a compound with glucuronic acid, and the resultant complex, sodium pregnanediol glucuronide, can be relatively easily isolated and estimated by direct weighing. The complete absence of pregnanediol in pregnancy is nearly always evidence of serious abnormality, suggesting in early pregnancy the imminence of abortion and in late pregnancy the death of the foetus, though the significance of a low pregnanediol excretion still remains uncertain and a normal excretion is not evidence that abortion will not occur or that the foetus is still alive. The method of estimation consists essentially in extracting the urine (1000 ml. or the 24 hours' output, whichever is less, before the twentieth week of pregnancy, and 500 ml. in the second half of pregnancy) with butyl alcohol, distilling off the alcohol and dissolving the residue in N/10 soda. This solution is again extracted with butyl alcohol, which is distilled off. The residue from this second distillation is dissolved in a small volume of water and the pregnanediol glucuronide precipitated by addition of excess of acetone. This precipitate is again dissolved in a small quantity of water and reprecipitated by acetone, the product thus purified being finally dried and weighed. The excretion is expressed in terms of milligrams of actual pregnanediol, this being 320/536 of the weight of the glucuronide complex. The total amount of pregnanediol excreted during the whole luteal phase of normal menstruation varies from 3 to 60 mg. Maximal excretion usually occurs 5 to 7 days before the onset of bleeding and may be from 1 to 6 mg. a day; it disappears from the urine before bleeding starts, but if pregnancy occurs and the period is suppressed, then excretion continues. The weight to which excretion rises varies considerably in different pregnancies, recorded figures varying from 30 to 120 mg. a day in the last six weeks of pregnancy.—C. L. Cope, *Brit. med. J.*, ii/1940, 545.

It is assumed that the liver is the site of conversion of progesterone. The pregnanediol glucuronide is not readily excreted in the presence of renal damage and therefore any assays of pregnanediol in the urine must take into account the presence of both hepatic and renal inefficiency. A quantitative estimation of pregnanediol may be used as an index of corpus luteum function, but the range of normality is wide. A complete absence of pregnanediol during the cycle is presumptive evidence of the failure of ovulation and may be taken as indicating a definite abnormality. In early pregnancy it suggests that abortion will occur and in late pregnancy foetal death.—Hadfield and Garrod, *Recent Advances in Pathology* (4th Edn.).

Owing to its limitations and to the amount of work that each analysis involves the test cannot supplant the Aschheim-Zondek or Friedmann tests, but is a useful adjunct to these and provides information both in the pregnant and in the non-pregnant woman that neither of these tests can give.—A. M. Hain, *Brit. med. J.*, ii/1940, 805.

Vitamins. The estimation of the urinary excretion of vitamins is of interest in assessing the presence and the degree of deficiency of a particular vitamin and in controlling its therapeutic administration. For the majority of the vitamins there are at present few

satisfactory methods available, but the following processes for the determination of ascorbic acid and vitamin B₁ have been found of value.

Ascorbic Acid. The urinary excretion of ascorbic acid by an adult on a full diet is about 25 mg. per day.

Harris and Ray Modification of Tillman's Method. Fill with urine a 2 ml. micro-burette reading to the nearest 0.01 ml. Measure 0.05 ml. of an aqueous solution of 2:6-dichlorophenolindophenol (standardised by titration with a solution of pure ascorbic acid so that 0.5 ml. is equivalent to 0.025 mg. of ascorbic acid) into a centrifuge tube, add 2 drops of glacial acetic acid and titrate with urine; the titration must be completed within two minutes. Titrate to the same colour as untreated urine of the same dilution in another tube. If more than 2 ml. of undiluted urine is required, the urine may be considered free of ascorbic acid. If less than 0.5 ml. is required, e.g., following ascorbic acid administration, the urine should be diluted and other titrations made. It is important that the urine be titrated immediately after passing, though acidification of the urine with 10% of its volume of glacial acetic acid permits of a delay of a few hours. The oxidised form may be reduced to the titratable form by sulphuretted hydrogen, the latter being removed by carbon dioxide, and this treatment should always be applied to urines that have been kept for more than a few hours. Regeneration with sulphuretted hydrogen is not effective after 18 hours.

It is difficult to diagnose scurvy or a sub-scurvy state by examination of isolated specimens of urine. The excretion after administration of daily doses of 400 mg. is a better guide.—H. E. Archer and Graham, *Lancet*, 1/1936, 710. Alternatively, a single dose of 600 mg. may be given.—Abbay *et al.*, *Arch. Dis. Childh.*, 1935, 327.

Alternative Method. It is confirmed that mercuric acetate removes from urine certain substances which interfere with the indophenol titration method for determining ascorbic acid, but reducing substances other than ascorbic acid are still present. Prolonged treatment with H₂S increases the reducing power of urine and so also does hydrolysis followed by reduction; these increases are probably due to the formation of ascorbic acid from the reduced form, i.e., dehydro-ascorbic acid, and from other derivatives. The following method of determination is suggested. The urine is acidified with 10% v/v of glacial acetic acid; if the urine is not used fresh, the acid serves as a preservative. 20 ml. of the sample is mixed with 20 ml. of water and 20 ml. of 20% mercuric acetate solution, the mixture is centrifuged and H₂S is passed through the supernatant liquid for 15 minutes. The liquid is filtered and CO₂ is passed through the filtrate for about 30 minutes to remove excess of H₂S. The filtrate is refluxed under CO₂ for 24 hours, diluted to 75 ml., and treated with H₂S for 96 hours. To 10 ml. of the liquid add 10 ml. of water and 10 ml. of 20% mercuric acetate solution. Centrifuge and remove Hg as before, and titrate for total indophenol-reducing substances. A further 20 ml. of the liquid is placed in an aeration tube and adjusted to pH 6.0 with 20% NaOH. 4 ml. of cauliflower "hexoxidose" solution (prepared as described below) is added and 10 ml. of mixture is immediately removed into 2 ml. of freshly prepared 50% metaphosphoric acid (specimen A). The remainder is allowed to stand for 30 minutes and a further 10 ml. is removed into 2 ml. of the metaphosphoric acid (specimen B). Both samples are then centrifuged and titrated. The indophenol reducing substances in B are due to substances other than ascorbic acid. The difference in the two titrations represents total ascorbic acid.

The enzyme solution is prepared as described by Hopkins and Morgan (*Biochem. J.*, 1936, 1446) from the florets and white stalks of the cauliflower by expressing the juice and diluting with the maximum amount (about 4 vols.) of water so as to effect complete oxidation of the ascorbic acid in 20 minutes.—H. Scarborough and C. P. Stewart, *Biochem. J.*, 1937, 2232.

Vitamin B₁. The daily output in urine of normal individuals varies between 50 and 170 µg., the mean value being about 100 µg. For the detection of deficiency it is necessary to give test doses of aneurine.

Thiochrome Test. The principle of the thiochrome reaction is to transform the vitamin B₁ into its fluorescent derivative by means of an alkaline oxidation with ferricyanide and to measure the degree of fluorescence against a suitable standard. Details of the test are given under vitamin B₁, p. 740.

A simple estimate of the daily excretion is so closely related to the vitamin intake of the previous few days that it throws little light on the patient's past history of vitamin B₁ deficiency. Measurements of urinary excretion of vitamin B₁ after ingestion of a test dose show that the shortest period of urine collection in saturation tests is five hours. The rate of excretion is modified by the time required for the stomach to empty.—J. Mason Hills, *Biochem. J.*, 1939, 33, 1966.

Estimation of vitamin B₁ in urine by the thiochrome test.—Y. L. Wang and L. J. Harris, *Biochem. J.*, 1939, 1956.

Significance of Vitamin B₁ in Urine. The urinary excretion furnishes a useful guide to the state of nutrition of the subject with regard to vitamin B₁. The low values so often found in medical cases may probably be attributed to one or more of the following causes: (a) anorexia, (b) poor hospital diets, (c) raised metabolism (especially in fever) and wasting of body tissues, (d) faulty absorption or utilisation. The following provisional standards are suggested: A "resting level" of excretion of 10 to 20 i.u. and a response of upward of 30 i.u. after the standard test dose (350 i.u.) implies normal average nutrition. Excretions of less than 10 i.u. (resting level) and 15 i.u. (after test dose) seem sufficiently removed from the average range, or from the excretion corresponding with the *reputed optimum requirements*, to be classified as subnormal. Polyneuritic symptoms of nutritional origin have been found to be associated with resting levels of 3.5 i.u. and under (or below 2 i.u. in 4 cases out of 5), and with low responses to test dose.—L. J. Harris *et al.*, *Lancet*, i/1938, 539.

Nicotinic Acid. The normal range is 3–5 mg. in 24 hours. Low values occur in pellagra and anorexia. Heavy smokers appear to show an increase of nicotinic acid in urine. A method for the assessment of nicotinic acid in urine.—L. J. Harris and W. D. Raymond, *Biochem. J.*, 1939, 33, 2037.

BLOOD

Blood Volume. In healthy adults the average normal volume of whole blood is about 85 ml. per kilo bodyweight. The blood volume is markedly increased in polycythæmia and slightly in leukaemia, in conditions associated with splenomegaly, in some cases of œdema, and during the later months of pregnancy. It is decreased in anæmia, severe shock, in pulmonary œdema due to war-gas poisoning and in conditions associated with dehydration.

Methods of determining blood volume in use at the present time are based on the principle that, by the addition of a definite quantity of a known substance to the circulation, the total quantity of blood may be calculated from the concentration of the foreign substance in a sample of blood. The carbon monoxide and the congo red method, or a combination of the two, are now most generally employed. By the former method the saturation of the red cells of an individual to whom a certain amount of carbon monoxide has been administered is determined, and the total quantity of blood is estimated from the relative quantities of red cells and plasma as determined by means of the hæmatocrit.

Method of Keith, Rowntree and Geraghty. Prepare a 1.5% solution of congo red in distilled water, filter and sterilise. Withdraw 10 ml. of blood and put into an oxalate tube. Inject the dye intravenously through the same needle, the dose being determined by dividing the bodyweight in kilograms by four. Four minutes later withdraw 10 ml. of blood from the other arm and put in an oxalate tube. Centrifuge both specimens in graduated tubes until the cells are packed; read the volume of the cells and plasma in both tubes and take the average. To 2 ml. of plasma from the first tube add 4 ml. of 0.9% sodium chloride solution and 2 ml. of a 1 to 100 dilution of the 1.5% solution of

the dye (this is used as the standard). To 2 ml. of plasma from the second tube add 6 ml. of 0.9% salt solution. Read in a colorimeter and calculate:

Plasma volume in ml. = $\frac{\text{No. of ml. of dye soln. injected} \times \frac{\text{Reading of unknown}}{\text{Reading of Standard}} \times 200}{\text{Reading of Standard}}$

Blood volume (ml.) = $\frac{\text{Plasma volume (ml.)} \times 100}{\text{Plasma volume per cent.}}$

The Hämatocrit is a simple and reliable method of determining the average volume of the red cells. There are many methods employed, but the most satisfactory is that of Wintrobe. In this a narrow glass tube of even bore, and etched with a centimetre-millimetre scale 10 cm. in length, is filled exactly to the 10 mark with oxalated venous blood. (10 mg. of potassium oxalate will prevent the coagulation of 5 ml. of blood. This causes a shrinkage in cell volume of approximately 5% which must be corrected for in the final result.) The tube is then centrifuged at 3000 revolutions per minute for about 30 minutes, and the volume of packed red cells read off the scale. This is normally 42.4 ml. per 100 ml. of blood. Further, if a total red cell count is performed at the same time it is possible to determine the average corpuscular volume, which is normally 80 cu. microns. For a more detailed description and an account of the other factors which may be deduced, such as the volume index, the mean corpuscular thickness and the mean corpuscular volume, the reader is referred to a paper in the *American Journal of Clinical Pathology*, i/1931, 147.

The Halometer devised by Young, is based on the diffraction method of measuring small particles and although the measurements by this method are readily performed, they yield average figures only and therefore afford no information which cannot be derived as readily and much more accurately from a hæmatocrit.

Reliability of the halometer.—A. Pijper, *Lancet*, i/1934, 483.

A comparative study of red cell diameter and red cell volume measurements.—J. M. Vaughan and H. M. Goddard, *Lancet*, i/1934, 513.

Estimation of Corpuscles

One cubic millimetre of blood contains normally about 5,000,000 to 6,000,000 red corpuscles in man, and about 4,500,000 in woman. The average number of white corpuscles per cubic millimetre is 5000 to 10,000 in adults; the range in children is somewhat higher.

The hæmocytometer chiefly used is the Thoma-Zeiss or Thoma-Hawksley. This consists of a micrometer slide divided into 16 squares, each again being divided into 16 smaller squares. It has two pipettes, one for diluting the blood 1 to 100, for counting the red corpuscles, while the other which dilutes the blood 10 times is intended for estimation of the leucocytes. The number of red corpuscles seen in 4, 6, or if great accuracy is required, 16 (larger) squares, i.e., in 64, 96 or 256 smaller squares, is counted.

Enumeration of Red Corpuscles. To ascertain the number in 1 cu. mm. of blood, knowing the volume of the cube standing on each small square to be $\frac{1}{16} \times \frac{1}{16} \times \frac{1}{16}$ cu. mm., multiply the total number of red corpuscles counted by 4000 times the number of times of dilution of the blood, and divide the result by the number of small squares in which red corpuscles have been counted. It is desirable to count the corpuscles touching and overlapping the two adjacent boundary lines on the left upper corners of the squares, but those on or overlapping the other two sides are excluded to compensate.

The normal dilution is 1 to 200; in polycythæmia 1 to 400; and in excessive anæmia 1 to 100 may be used. 5 or 6 corpuscles per square are a convenient number for counting.

The Thoma-Zeiss cell is $\frac{1}{16}$ mm. deep and each side of a small square is $\frac{1}{16}$ mm., hence the above figure $\frac{1}{16} \times \frac{1}{16} \times \frac{1}{16}$ cu. mm. as the volume of a small square.

The Burk counting chamber, in which there are two rulings separated by an H-shaped moat, greatly facilitates filling the chamber, and the modified Neubauer ruling makes the actual counting much quicker and easier.

Gowers' Hæmocytometer Solution is still used as a diluent. Sodium sulphate 5.42 g., acetic acid 3.125 ml., distilled water 100 ml. Filter.

Hayem's Solution is also employed. Sodium chloride 2, sodium sulphate 5 mercuric chloride 0.5, water 200.

Toison's Solution (see below) is also employed. It stains the leucocytes.

Wright's Diluting Fluid for counting red corpuscles. Sodium chloride 1, mercuric chloride 0.2, distilled water to 100.

The "**Colour Index**" is the index of corpuscular richness. It is obtained by dividing the percentage of hæmoglobin by the percentage of red corpuscles. With the normal of red corpuscles as 5,000,000 and the hæmoglobin at 100 the index $\frac{100}{5000000} = 1$. In a case of red corpuscles 4,000,000 (=80% of normal) and hæmoglobin 40%, the index would be $\frac{40}{4000000} = 0.5$.

Enumeration of Leucocytes may be conducted in a similar manner, by the Thoma-Zeiss instrument, but in this case it is desirable to stain them before counting by using Gowers' diluting fluid with an appreciable addition of Löffler's methylene blue, or by Toison's Solution. (Dissolve methyl violet 5 B. 0.025 g. in a mixture of glycerin 30 ml. and water 80 ml. Dissolve separately sodium sulphate 8 g. with sodium chloride 1 g. in water 80 ml. Mix and filter.) Leucocytes stain violet, red corpuscles greenish. For accuracy, count as many squares as possible.

A further formula for the staining fluid is solution of formaldehyde 1.5, sodium chloride 0.5, sodium sulphate 2.5, methyl violet 0.01, water 100.

Another method is to use a 3% aqueous acetic acid solution tinted with methylene blue or gentian violet as diluent; in this the red corpuscles become invisible while the leucocytes remain visible, and the blood need only be diluted 1:10 thus increasing the accuracy of the count.

The following is a simple method of counting. To stain, a 3% sodium chloride solution deeply coloured with gentian violet is sufficient. It is simpler to count whole microscopic fields of known area rather than squares. Employing the 1 in 20 pipette, count whole microscopic field, not the squares, move the draw-tube of microscope into such position that $7\frac{1}{2}$ squares in diameter (Thoma-Zeiss scale) are in view. The cubic contents of this = 1.75 cu. mm. Make a mark on the draw-tube—to be used for all occasions. Count twenty fields with above dilution, and add two cyphers to the number so obtained.

Enumeration of the Blood Platelets. These may be counted directly or indirectly; in the latter case the proportion of platelets to red cells is determined and from a red cell count performed at the same time the absolute platelet count is calculated. The diluting fluids used in both methods are designed to prevent adhesion and disintegration of the platelets. The normal platelet figure is 200,000 to 400,000 per cu. mm.

Lesspert's Direct Method. Two solutions "A" and "B" are employed. "A" contains sodium citrate 1.0 g., mercuric chloride 0.002 g., brilliant cresyl blue 0.2 g. dissolved in 100 ml. of distilled water at 45°. "B" is made up of urea 20 g. dissolved in 100 ml. of distilled water. Equal amounts of "A" and "B" are mixed when the diluting fluid is required; the mixture is centrifuged for 5 minutes and filtered prior to use. Some of it is drawn up to mark 0.5 on the stem of the leucocyte pipette, then blood to mark I, followed by mixture again to mark II. The resulting blood dilution is 1 in 20. The pipette is shaken briskly and a drop of the fluid mounted on a Thoma-Zeiss hæmocytometer. The red cells are hæmolyzed by the urea in the diluent. The number of platelets in 80 small squares is counted; this figure $\times 1000$ = number per cu. mm.

Rees and Eckers Indirect Method. The finger is pricked through a drop of the special diluting fluid (sodium citrate, 3.8 g.; formalin, 0.2 ml.; brilliant cresyl blue, 0.1 g.; distilled water, 100 ml.; filter before using) and a drop of the diluted blood is placed on a slide, covered with a cover slip and examined wet and unfixed.

Total and Differential Count Simultaneously. By using Kristenson's fluid it is possible to perform a red and white count, a differential white count, and a platelet count with one specimen of blood. The blood is diluted (1:20) in a pipette in the ordinary way with the following fluid:—sodium citrate 2.5 g., mercuric chloride 0.005 g., brilliant cresyl blue 0.5 g., water 250 ml. Before use it is mixed with an equal quantity of aqueous 5% urea. The leucocytes are so well stained that with a little practice a reasonably accurate differential count may be performed, but the platelet count will tend to be lower than it actually is owing to the adhesion of platelets to the wall of the pipette.

Hæmoglobin Determination

The average hæmoglobin content of normal blood is 14·5 g. per cent. This corresponds with a percentage of 105 on the Haldane scale and 85 on the Sahli scale. The direct method with the Lovibond comparator reads on the Haldane scale.

Although the determination of the amount of hæmoglobin is one of the most important of all the chemical tests of the blood, yet as a rule it is the one which is determined with less care and by methods more inaccurate than those in use for any other constituent of the body. There are three methods in common use.

Carbon Monoxide Method. The Haldane modification of Gowers' method is the best known. The graduated tube is filled to the mark 10 with distilled water and 20 cu. mm. of blood added. The hæmoglobin is then converted into carboxyhæmoglobin by allowing a stream of coal gas to play over the diluted blood for a few minutes. Water is then added drop by drop until the tint of the standard tube is matched. The standards tend to fade and it is as well to have the instrument checked occasionally.

Acid Hæmatin Method. In the Sahli hæmoglobinometer and its modifications (Hellige, Newcomer, etc.) the blood is diluted with N/10 hydrochloric acid; this converts the hæmoglobin into acid hæmatin, which is a brownish suspension. This colour is more easy to match than that of whole blood and the necessity for a supply of coal gas (one of the disadvantages of the Haldane apparatus) is avoided.

The actual method of diluting the blood varies with different models. In some the graduated tube is filled to the mark 10 with N/10 hydrochloric acid, and 20 cu. mm. of blood collected in a pipette is added; then distilled water is added drop by drop until the tint of the standard tube is matched. In others the blood is collected in a special diluting pipette which is filled to the mark 1 with blood and then filled up to the mark 101 with N/10 hydrochloric acid, the pipette is then well shaken to ensure proper mixing and the contents of the pipette are run into a special rectangular cell. The standard is a movable wedge which is adjusted until the tints match.

Originally the standard was a solution of acid hæmatin, but this was found to fade badly and most instruments now employ a rod or wedge of coloured glass which is said to be permanent in colour.

It is important to remember that the colour of the solution of acid hæmatin gradually increases in intensity, and accordingly the reading should be made at a standard time after collection of the sample. (About 95% of the colour is attained in 10 minutes and after one-half to one hour the change is insignificant.) Further, as in all colour-matching procedures, readings should be made with a uniform source of light and, in making comparisons, several rapid readings should be made and the average of these taken; otherwise the eye will become fatigued and the reading will be erroneous.

Direct Method. This was the original method employed by Gowers and it is at present used in the Dare hæmoglobinometer and in the estimation of hæmoglobin with the Lovibond comparator. In both these a thin film of undiluted blood is compared with a glass disc of graduated colour under uniform lighting. A reading can be obtained very rapidly, but the instruments are expensive. The Lovibond comparator gives the more accurate reading. —G. A. Harrison, *Lancet*, ii/1938, 621.

A photoelectric spectrohæmoglobinometer has been devised which gives very accurate results (*J. Lab. clin. Med.*, 1930, 15, 483), but its use is still limited to the laboratory, and the same applies to the oxygen capacity method of Van Slyke and the quantitative estimation of the iron content of the blood. —*J. biol. Chem.*, 1927, 74, 385.

Sulphæmoglobinæmia and Methæmoglobinæmia. These pigments appear as intracorporeal derivatives of hæmoglobin after the prolonged administration of drugs derived from aniline or nitrobenzene. Of these drugs the most important are phenacetin (I. Snapper, *Nederlandsch tijdschrift voor geneeskunde*, 1922, 66, 2541), acetanilide, and the more recently introduced sulphanilamide (*p*-aminobenzenesulphonamide) (Colebrook and Kenny, *Lancet*, i/1936, 1279; Discombe, *Lancet*, i/1937, 626; Archer and Discombe,

Lancet, ii/1937, 432). The pigments are readily detected spectroscopically in laked blood and may be seen in the circulating blood by transillumination of the lobe of the ear.

Methæmoglobin rapidly disappears with withdrawal of the drug. Sulphæmoglobin may persist for several weeks. The development of sulphæmoglobin is due to the absorption of H_2S from the bowel and this can be prevented to a great extent by keeping the colon free from food residues, by avoidance of purgatives, and by giving a low residue diet containing very few eggs. Regular doses of liquid paraffin are advisable. Cyanosis is the usual indication of the presence of these pigments. It occurs when about 5 g. of hæmoglobin per 100 ml. of blood is in the reduced form or in the form of sulphæmoglobin or methæmoglobin. It follows that a patient with a severe anæmia and a hæmoglobin content of 30% cannot become cyanosed although there may be sulphæmoglobinæmia or methæmoglobinæmia. Spectroscopic examination of the blood is therefore essential in excluding the condition. Extracorporeal methæmoglobinæmia has been noted in severe sepsis, transfusion of incompatible blood, blackwater fever, quinine poisoning and phenylhydrazine poisoning. It is accompanied by methæmoglobinuria. Hæmolysis is present and there may be jaundice. For methods of distinguishing methæmoglobin and sulphæmoglobin see Harrison.

Preparation of Blood Films

To make films, prick patient's finger, press, let first drop of blood fall away, place the next drop (small) on the centre of a *really clean* grease-free $\frac{3}{4}$ in. square cover slip. Superimpose another and slide off so that the film is thin and even—not "ridges" and "valleys"—and dry in the air. No fixing is necessary, the methyl alcohol in the stain (Leishman, etc.) does this.

Films may also be made on 3×1 in. glass slides. On the narrow edge of one slide is taken up a small drop of blood. This edge is then applied to the surface of a clean grease-free slide which is lying flat on a firm smooth surface. The blood will spread across the edge and a thin film is made by pushing the drop along the clean slide with the "spreading" slide held at an angle of about 45 degrees.

To Clean Cover Slips and Slides. Place the cover slips in pure nitric acid for 24 hours, stirring occasionally to ensure that the acid comes in contact with all the slips, wash in running water for the same time and keep in a well-stoppered bottle in spirit until required. Just before use the requisite number are removed from the spirit with forceps and dried either by burning the spirit off or by polishing with a piece of well washed linen.

The older methods of staining the films (Ehrlich's Triacid, Ehrlich's Triple Stain and Jenner's Stain) have been entirely superseded by Leishman's Stain and its modifications.

Romanowsky's Stain, Leishman's Modification. There are various modes of making and applying this stain. The following as suggested by Leishman gives the best results (the fixing and staining is done in one process so that fixing by heat is unnecessary):—

This is a solution in pure methyl alcohol of an eosin-methylene-blue-precipitation compound, 0.15 g. of the compound being dissolved in 100 ml. of methyl alcohol. (The methyl alcohol must be neutral in reaction and acetone-free.) The solution thus formed is a clear dark-blue liquid showing a green iridescence by reflected light. The stain is used by preparing films of blood in the usual way on clean cover glasses or slides, and allowing to dry in the air. The films should be as thin as possible. Three or four drops of the stain are dropped on to the film and the cover glass is rotated, no attempt being made to check evaporation. After about half a minute six or eight drops of distilled water

are added, and allowed to mix by rotating with the stain, and staining is allowed to proceed for 5 minutes; in certain cases 10 minutes may be necessary. The film is now washed with distilled water, and a few drops of the water are allowed to remain on it for 1 minute. It is finally dried without heating and examined with an oil immersion lens.

The following results should be obtained:—Nuclei are stained a reddish-purple, cytoplasm tends to be very pale blue, neutrophile granules are a dirty pink, eosinophilic ones copper-red, basophilic ones metachromatic purple, and azurophilia is shown by a cherry-red colour, but only with specimens of the stain that have been stored for some time. Red corpuscles are copper-red unless they are polychromatic, when they are a dirty pink, but slight degrees of this are not shown by this stain. Blood platelets are purplish with reddish granules, malarial parasites stain blue with red chromatin granules (*vide also Malaria, this volume, p. 925*). Bacteria stain a light blue.

It is important that the distilled water employed should be neutral; if slightly acid, the eosinophile granules will stain very brightly but the nuclei will be very faint; if alkaline, the red cells will be bluish and the cytoplasmic granules will not be stained. If there is difficulty in obtaining a satisfactory result the Haden Buffer solution described below can be used to replace the distilled water with advantage.

Contrary to the generally held opinion acetone is *not* a detrimental substance in Romanowsky's stain. Actually it is useful as a solvent for blood stains and in some respects is superior to methyl alcohol; with a given dye concentration it permits more rapid staining than the latter. Experiments on the participation of solvents in the staining process indicate that the concentration of water is the most important variable in the solvent and not the presence of acetone.—D. M. Kingsley, *J. Lab. clin. Med.*, 1937, 524.

Pappenheim's Panoptic Method is to be recommended if very fine cytological detail is desired. Stain the air-dried films in May-Grunwald's stain face downwards in a covered vessel for 3 minutes; add an equal quantity of Haden's Buffer solution (6.63 g. of chemically pure crystalline potassium acid phosphate and 2.56 g. of anhydrous basic sodium phosphate dissolved in 1 litre of distilled water; this solution should have a pH of 6.4) and stain for 1 minute more; place the film without washing into the following solution: Haden's solution, 5 ml.; Panchrom (or Giemsa's stain), 5 drops; orange-G-methyl green stain, 2 drops. Stain in this mixture for 6 to 12 minutes. (The orange-G-methyl green stain is prepared as follows: Equal parts of 1% aqueous solutions of orange-G and methyl green are mixed. The heavy precipitate that results is collected, dried and dissolved to saturation in pure methyl alcohol, and constitutes the stain.) The films are then washed in Haden's solution for 30 seconds and dried at room temperature. Distilled water may be substituted for Haden's solution throughout the technique but the results are not so good.

Relative and Absolute Normal Leucocyte Counts (per cu. mm.)

| Type of Cell | Per cent. | Absolute Number | | |
|--------------------------|-----------|-----------------|---------|---------|
| | | Average | Minimum | Maximum |
| Total Leucocytes .. | | 7000 | 5000 | 10000 |
| Myelocytes | 0 | 0 | 0 | 0 |
| Juvenile neutrophiles .. | 4—8 | 400 | 200 | 700 |
| Segmented neutrophiles | 56—62 | 4200 | 2800 | 5800 |
| Eosinophiles | 1—3 | 200 | 50 | 300 |
| Basophiles | 0—0.75 | 35 | 15 | 75 |
| Lymphocytes | 20—30 | 2000 | 1000 | 3000 |
| Monocytes | 4—8 | 450 | 300 | 600 |

Blood counts. The conclusions of the orthodox hæmatologist when confronted by criticisms such as those of R. H. Simpson (*Brit. J. Radiol.*, 1933, 6, 705) and G. W. Phillips were summarised by Prof. Eric Ponder and his collaborators in 1931 (*Quart. J. exp. Physiol.*, 1931-32, 21, 35) as follows: "Under conditions of moderate activity the large fluctuations in the total white cell count described by Sabin, Cunningham, Doan and Kindwall have not been observed. Those large fluctuations seem to be due principally to errors of method. The total white cell count shows, however, small fluctuations not exceeding $\pm 8\%$ throughout the day; these persist even after all errors of method have been allowed for." Until overwhelming evidence is produced that Prof. Ponder is wrong, it is safer to continue to assume a significance in pronounced variations of leucocyte counts, especially when dealing with workers in radiological departments.—*Lancet*, ii/1933, 1098.

Cooke Polynuclear Count. W. E. Cooke has simplified and improved the original Arneth leucocytic index, and the method is often of value, particularly in judging the progress of a disease. One hundred consecutive neutrophile polymorphonuclears are counted and arranged in groups according to the number of nuclear lobes. These groups are then expressed as percentages of the neutrophiles, not of the total leucocytes. The normal figures for the number of cells in each class are as follows: Class I 10%, Class II 25%, Class III 47%, Class IV 16%, Class V 2%. For the significance of changes in the count and the exact definition of what is meant by a lobe see *The Polynuclear Count* by W. E. Cooke and Eric Ponder.

The Schilling Hæmogram divides the neutrophiles into myelocytes, metamyelocytes (nucleus indented or S-shaped, basi- and oxy-chromatin clearly differentiated), stab forms or juvenile neutrophiles (nucleus ribbon-like and twisted, or sausage-shaped, but pyknotic), and mature segmented forms. The numbers of the different types are then expressed as percentages of the total leucocytes in contrast to the Cooke index. The information obtained is different from that in the Cooke-Arneth index, as the more immature granular cells are studied in greater detail, but the number of lobes in the segmented forms is ignored, and certain hæmatologists have questioned the Cooke-Arneth criteria for judging the maturity of the neutrophile whereas Schilling's grouping is unquestioned.

Oxydase Reaction. In certain cases of leukaemia the oxydase reaction may be of assistance, but it is important to recognise that myeloblasts, lymphoblasts and all the cells of the lymphoid series are oxydase-negative, the promyelocyte, myelocyte and mature granular cells are oxydase-positive, and monocytes may be oxydase-negative or show a few granules in the cytoplasm. The most reliable method is that of Bryce. Two solutions are used: (A) 0.3 g. benzidine base, 1 ml. of a saturated aqueous solution of sodium nitroprusside, ethyl alcohol 100 ml. (B) 0.5% solution of hydrogen peroxide. Air-dried blood films are treated with the solution A for 1 minute, then an equal quantity of solution B is added and allowed to act for a further 3 minutes; the films are then washed for 10 minutes and allowed to dry in air. They may then be counterstained with Leishman, but the dilute stain should be allowed to act for at least 10 minutes.

Punctate Basophilia and Polychromasia. In cases of suspected lead poisoning it is often important to detect the slightest degree of punctate basophilia, and the ordinary eosin-methylene blue stains fail to do this; accordingly the method of Manson-Schwarz is employed. Two solutions are required: (A) boric acid 2 g., methylene blue 1 g., distilled water (carbon-dioxide-free) 100 ml.; (B) 0.28% sodium hydroxide in boiled distilled water. Immediately before use, 6 drops of solution A are mixed with 8 drops of solution B and made up to 10 ml. with boiled distilled water. Air-dried blood films are fixed in methyl alcohol for 5 minutes, washed with distilled water and then placed in the stain for 5 seconds; washed with distilled water, dried in air and mounted.

In normal persons the number of basophilic cells per ml. of blood is as a rule less than 1000 and never exceeds 5000. Lead poisoning produces counts over 7000 and up to 100,000, but symptoms may not occur even when the count is as high as 80,000 to 80,000. When a worker who is exposed to lead develops a basophilic red cell count over 6000 or 7000 and when other conditions which might produce such a count are absent (certain physiologic states—benzene poisoning, arsenic poisoning, all types of anaemia in which there is regeneration, hæmolytic icterus, the condition following hæmorrhage, leukaemias, acute infections, neoplasms involving bone marrow and polycythæmia—) increase the proportion of basophilic cells up to 20,000 the worker

should be considered a lead poisoning prospect.—*J. Amer. med. Ass.*, ii/1928, 251.

In any workman exposed to lead the presence in the blood film of erythroblasts, particularly if punctated, indicates that suspension from work is necessary. Punctate basophilia and cabot rings are never present in healthy blood.—*R. Craik, Brit. med. J.*, i/1937, 888.

Reticulocytes. The presence of reticulated red cells can only be determined in unfixed blood by means of supravital staining. There are normally 0.5 to 1% in the circulating blood, and their study is of value in judging the response to treatment in the anæmias. Perfectly clean grease-free slides should be slightly warmed, and a drop of a 0.3% solution of brilliant cresyl blue in absolute ethyl alcohol applied. If the slides are clean the stain will spread out in an even ring and dry quickly. (A large number may be prepared at one time and will keep indefinitely if kept dust-free.) When the count is to be made, a small drop of blood is collected on a grease-free cover slip and placed on the ring of stain on the slide. The cover slip is ringed round with soft paraffin to prevent drying, and may be examined in 10 minutes. The reticulated red cells are expressed as a percentage of the red cells.

Vital Staining of the Leucocytes by Janus green B and neutral red is a complicated laboratory procedure, and readers must be referred to the papers of Sabin and Simpson for details of the technique. (See *Bull. John Hopkins Hosp.*, 1923, 34, 277, *et seq.*).

Determination of the Size of the Red Cell. In the Price-Jones Curve the diameters of 500 red cells in a stained blood film are measured, either by projection on to a screen or by means of an eyepiece micrometer, and the results plotted in the form of a graph. From this may be determined the mean diameter of the red cells and the degree of variation in the size of individual cells. The normal mean diameter is 7.2 microns. The method is laborious but there is no other way of obtaining the information it gives. The determination is of value in the diagnosis of pernicious and secondary anæmias.

The average diameter of the red cells in a film can be determined simply and quickly, but with less precision, by means of Pijper's halometer (see p. 672). This does not determine the degree of anisocytosis, however, and if this is marked it is difficult to obtain precise readings.

Acid-base Balance of the Blood

The appearance of acetone bodies in the urine in appreciable quantity was originally taken as the index of "acidosis" (see *Urine Analysis*, p. 636), and, from the essentially practical point of view, acidosis due to the ketone bodies is still the most important clinically. Thus, in untreated diabetes with acetone bodies in the urine it may be assumed that there is an acidosis, but in dealing with conditions less well studied, it is now generally agreed that cases of disturbed acid-base balance are best diagnosed not by the determination of acetone bodies in the urine or blood, but by the determination of certain other factors more or less typical of "acidosis." These include the following determinations: (1) the "alkali reserve" of the blood; (2) the hydrogen-ion concentration of the blood; (3) the carbon dioxide tension of the alveolar air; (4) the alkali tolerance of the patient.

The main "alkali reserve" of the plasma is in the form of BHCO_3 (plasma bicarbonate). The acid absorbed into or formed within the body reacts with the BHCO_3 liberating CO_2 , which is promptly excreted by the lungs thus preventing any change in the pH of the blood. The pH of the blood depends directly on the ratio between the plasma bicarbonate and its CO_2 tension (this ratio is 1 to 19, with a normal pH of 7.4). If the H_2CO_3 content falls, the bicarbonate remaining constant, the blood will become

more alkaline, the pH will rise; if the bicarbonate content falls, the H_2CO_3 remaining constant, the blood will become more acid, the pH will fall. So long as bicarbonate and H_2CO_3 both alter proportionately and in the same direction the pH will not alter. An estimate of only one of the three variables, i.e., bicarbonate content, H_2CO_3 content and pH, does not permit of the forming of a definite opinion as to the real condition of the patient. For this purpose two of the three must be determined, and for this reason these methods are not commonly employed in clinical work. At the same time in the commoner types of disturbance of acid-base equilibrium, determination of the "alkali reserve" alone is a valuable procedure.

Determination of "alkali reserve." Oxalated blood collected under liquid paraffin is shaken with air whose carbon dioxide tension approximates that of normal arterial blood, by which it combines with as much carbon dioxide as it is able to hold under normal tension. A known quantity of the saturated plasma is then acidified within a suitable pipette and its carbon dioxide liberated by the production of a known partial vacuum. The volume of the liberated carbon dioxide is then measured at atmospheric pressure and the volume corresponding to 100 ml. of plasma calculated. (For further details of this method together with a description of the Van Slyke carbon dioxide apparatus and a table for the calculation of the carbon dioxide combining power of plasma see Harrison; other methods of determination are given by Hawk.)

Determination of Hydrogen-ion Concentration. Various colorimetric methods are available (Hawk). In that of Cullen, as modified by Hawkins, the blood is collected with precautions against the loss of CO_2 , the plasma is diluted with saline solution containing phenol red, and the colour obtained compared with that of standard phosphate mixtures of known pH.

Alkali Tolerance Test (Sellard's Test). This depends on the ability of a normal individual to secrete an alkaline urine after taking 5 to 10 g. of sodium bicarbonate. In acidosis, the excess of acids combines with the alkali, forming a neutral salt, and much larger doses have therefore to be given. The reaction of the urine is tested with litmus paper 1 hour after successive 5 g. doses of sodium bicarbonate, the urine being first boiled and cooled. The amount of alkali required to render the urine alkaline gives an indication of the degree of acidosis, 30 to 60 g. being necessary in moderate cases.

Blood Coagulation

The processes involved in the coagulation of the blood are not fully understood and several theories have been advanced. The theory which appears to be as firmly founded upon experimental evidence as any is substantially the following (Hawk). The blood remains fluid in the vessels due to the absence of some factor essential for coagulation. This important factor is *prothrombin* which is combined in circulating blood with a protective or inhibiting substance called *heparin*. When blood is shed the tissue factor *thromboplastin* or *cephalin*, which is liberated from disintegrated platelets (or wounded tissue-cells), promptly unites with heparin and the prothrombin is released. In the presence of blood calcium the prothrombin is then transformed into an enzyme called *thrombin*, and this in turn acts upon the soluble *fibrinogen* to produce insoluble *fibrin*. This fibrin forms in shreds throughout the blood mass and, holding the elements of the blood within its meshes, serves to produce the typical *blood clot*. The fibrin shreds gradually contract (clot retraction), the clot assumes a jelly-like appearance and the yellowish *serum* exudes.

If, immediately upon the withdrawal of blood from the body, the fluid be rapidly stirred or thoroughly "whipped" with a bundle of coarse strings, twigs or a specially constructed beater, the fibrin shreds will not form in a network throughout the blood mass, but instead, will cling to the device used in beating. In this way the fibrin may be removed and the remaining fluid is termed *defibrinated blood*. The above theory of the coagulation of the blood, apart from the relation of heparin to the process, may be stated briefly as follows:

I. Prothrombin + Thromboplastin (or cephalin) + Calcium = Thrombin.

II. Thrombin + Fibrinogen = Fibrin.

Recently it has been shown that vitamin K (*see p. 763*) is essential to prothrombin production and is thus of vital importance in the mechanism of blood coagulation. If there is inadequate intake of this vitamin in the diet, or if its absorption is impaired, the blood content of prothrombin falls to a low level, resulting in an increase in the clotting-time of the blood. The usual coagulation tests reveal only serious blood abnormality or dangerous prothrombin levels, and there is at present no method of measuring directly the amount of prothrombin in the blood to enable a diagnosis of vitamin K deficiency to be made or to assess the results of vitamin K therapy. A fairly accurate determination may, however, be made by Quick's Method (*vide infra*).

Coagulation Time (Dale and Laidlaw). The Dale and Laidlaw Coagulation Tube consists of a standard sized capillary tube which is slightly lipped at each end to allow free entry of blood, and to retain a small lead shot which is free to roll from one end of the tube to the other. The tube is filled with blood by capillary attraction and transferred to a water-bath at 37° where it is held by a special pair of forceps which occlude the open ends. It is then tilted up and down until the travel of the lead shot is just arrested by the process of coagulation. The time taken between the first appearance of the blood and the arrest of the lead shot is the coagulation time and is normally two minutes by this method. It is greatly prolonged in hæmophilia. A rather more satisfactory instrument is Gibb's Coagulometer described in the *Quarterly Journal of Medicine*, 1923-4, 17, 312.

Quick's Prothrombin Test. Mix 4.5 ml. of venous blood obtained (by venipuncture) immediately with 0.5 ml. of oxalate reagent and centrifuge to obtain plasma. Add to 0.1 ml. of plasma, 0.1 ml. of thromboplastin solution and 0.1 ml. of calcium reagent. Record accurately the time required for the formation of a clot after the addition of calcium. Normal plasma clots in 11 to 12 seconds and the clotting time is prolonged with a decrease in prothrombin as shown in the table below:—

| Clotting Time in Seconds. | Concentration of Prothrombin in Plasma (Human) per cent. |
|---------------------------|--|
| 11-12 | 100 |
| 12½ | 80 |
| 13½ | 60 |
| 15 | 50 |
| 17 | 40 |
| 19½ | 30 |
| 22 | 25 |
| 25-26 | 20 |
| 37-40 | 10 |
| 55-65 | 5 |

The reagents used are:—(1) *Sodium Oxalate* (0.1 molar solution): Dissolve 1.34 g. of anhydrous pure sodium oxalate in 100 ml. of distilled water. (2) *Calcium Chloride* (0.025 molar solution): Dissolve 1.11 g. of anhydrous pure calcium chloride in 400 ml. of distilled water. (3) *Thromboplastin Solution*: Mix 0.3 g. of dehydrated rabbit brain with 5 ml. of physiologic solution of sodium chloride containing 0.1 ml. of sodium oxalate. Incubate at 45° for 10 minutes; centrifuge at slow speed for 3 minutes to obtain a milky supernatant liquid free from coarse particles.

Other methods, which are modifications of the original test, are Smith's Two-Stage Research Method and Kato's Micro-Prothrombin Test (see *Amer. Prof. Pharm.*, 1940, 701); a simple bedside test is described in Vol. I of this edition, p. 25.

Prothrombin determination using **heparin** as anticoagulant.—H. Dam and J. Glavind, *J. Amer. med. Ass.*, ii/1940, 150.

The estimation of prothrombin is much simplified by the use of a preparation of **Russell-viper venom** as the thrombokinase. The results are similar to those obtained with tissue extracts.—H. W. Fullerton, *Lancet*, ii/1940, 195.

The coagulant action of Russell-viper venom on blood and plasma can be potentiated by the addition of lecithin. Observations on the use of this venom alone and **venom plus lecithin** as the clot-accelerating reagents in Quick's test suggest that this technique allows us to recognise three types of hæmorrhagic tendency. The method for substituting venom or venom plus lecithin for the brain extract in Quick's test is as follows: 9 ml. of blood, withdrawn rapidly and with special precaution to avoid trauma, are promptly and thoroughly mixed with 1 ml. of M/10 sodium oxalate and centrifuged at a low speed for five minutes. Of this plasma 0.1 ml. is transferred to a clean dry test-tube (13 × 100 mm.) in a water-bath kept at 37° and mixed with 0.1 ml. of venom or venom plus lecithin. Without delay 0.1 ml. of M/40 calcium chloride is added, the tube quickly shaken and the exact time required for the formation of a solid clot recorded. Under the conditions of Quick's test the optimum amount of lecithin is about 5 mg. per ml. of venom. This concentration is obtained by adding 0.05 ml. of a 10% alcoholic solution of ovolecithin to every ml. of the solution of venom. In hæmophilia the blood coagulates like that of normal people when snake venom is added. In purpura hæmorrhagica and certain hereditary hæmorrhagic states coagulation may be less rapid than normal when venom alone is used but is not usually different from normal when venom and lecithin are added. In prothrombin deficiency coagulation remains impaired despite the addition of venom plus lecithin. If venom is used, instead of brain extract, to measure prothrombin in the Quick test it must be fortified by the addition of lecithin.—L. J. Witts and F. C. G. Hobson, *Brit. med. J.*, ii/1940, 862.

The Quick prothrombin test is merely a measure of the accelerated clotting time of the blood plasma under conditions designed to exclude hæmophilia. Workers with this and other tests must constantly remind themselves that what they are measuring is the ability of the blood to clot and that translation of the results in terms of prothrombin implies assumptions which are not always justifiable. Thus, no relationship can be demonstrated between the level of prothrombin in the mother and her newborn child and this suggests that we cannot pre-select mothers for prophylactic treatment with vitamin K. It has been shown that the accelerated clotting time of blood from the umbilical vein is shorter than that from the artery and it has been suggested that prothrombin may actually be formed by the placenta during gestation and that the fall in the prothrombin level of some infants after birth may be due in part to the removal of such a source of supply.—per *Brit. med. J.*, i/1942, 154.

Quick's prothrombin test is based on the hypothesis that if the coagulation of plasma is accelerated by excess of thromboplastin the speed of coagulation will depend mainly on the amount of prothrombin present. Other things being equal the speed of coagulation is inversely proportional to the concentration of prothrombin. When reagents composed of mixtures of Russell-viper venom and crude lecithin are used as thromboplastins the coagulation time of recalcified plasma can be reduced to 5 seconds. Unless instrumental methods of recording are used such reagents are too swift for accurate work. The venom-lecithin reagent has been standardised to give an average coagulation time of 10 seconds. The normal range of variation has been determined and methods of scoring the results in terms of prothrombin are discussed.—L. J. Witts and F. C. G. Hobson, *Brit. med. J.*, i/1942, 575.

Test for Vitamin K Deficiency. With a serologic pipette 0.1 ml. of thromboplastin (*see below*) is placed in a small serologic tube (75 x 10 mm. outside diameter). In the tube is then placed blood, freshly drawn from the patient, up to a 1 ml. mark previously made on the side of the tube. The tube is at once inverted over the finger to obtain complete mixing of the blood and thromboplastin. The tube is tilted every second or two in order to observe clotting. As a control, the test is also carried out on the blood of a normal subject. The calculation is as follows:—

Clotting activity $\frac{\text{Clotting time of normal control} \times 100}{(\text{in percentage of normal}) = \text{Clotting time of patient's blood}}$

Thus, if the patient's blood clotted in 48 seconds and the normal person's blood in 24 seconds the clotting activity is calculated to be 50% of normal.

To prepare *thromboplastin*, fresh lung of ox or rabbit is ground and to each 10 g. portion is added 10 ml. of normal saline solution. This is stirred at intervals for several hours. The fluid then obtained by straining through gauze is the thromboplastin. Experience with a large group of patients having biliary fistulas or obstructive jaundice shows that bleeding commonly occurs when the test gives values of 40% or less. In extreme cases the level falls as low as 15% of normal. Values of from 40 to 70% are definitely in the danger zone. Vitamin K deficiency is to be expected in all cases in which there are biliary fistulas or obstructive jaundice. If a deficiency does exist it is important that the patient receives vitamin K therapy several days before any operative procedures are undertaken.—H. P. Smith *et al.*, *J. Amer. med. Ass.*, ii/1939, 380.

Bleeding Time (Duke's Method). The lobe of the ear or finger is pricked so that the blood flows easily drop by drop without any assistance; each drop is removed with filter paper as it forms until the bleeding stops, care being taken not to touch the skin. The time interval between the appearance of the first drop and the removal of the last represents the bleeding time. Normally it is two or three minutes and is greatly prolonged in most purpuric conditions.

Capillary Resistance Test (Rumpel-Leede Phenomenon). If a sphygmomanometer arm band adjusted to a pressure just above the diastolic is left on the arm for 3 to 10 minutes, a crop of petechiæ will appear distal to the tourniquet in those hæmorrhagic states in which there is abnormal capillary permeability. The test is of value in the detection of vitamin C deficiency (*see this volume*, p. 754).

Fragility of the Red Cells. The resistance of the red cells to hypotonic salt solutions is usually determined by the method of Sanford. Twelve small test-tubes are set up, and into each is placed an equal amount of a solution of sodium chloride ranging from 0.3 to 0.9%, each tube differing by 0.05%. A drop of blood is put into each tube and mixed with the salt solution. In 2 hours the results are read. Commencing hæmolysis is indicated by a slight reddish colouring which has resulted from the laking of the least resistant cells. Complete hæmolysis is indicated by a clear red solution and the absence of any corpuscular residue on shaking the tube. Normally hæmolysis commences at 0.45% and is complete at 0.35%. Increased fragility is found notably in hæmolytic jaundice, and decreased fragility after splenectomy, especially for diseases other than alcoholic jaundice.

Blood Sedimentation Rate

This investigation is of value in assessing the activity of an infection, particularly tuberculosis, chronic rheumatism and rheumatoid arthritis. The usual technique employed is that of Westergren. 1.6 ml. of blood is drawn into a syringe containing 0.4 ml. of 3.8% sodium citrate as anticoagulant. This mixture is emptied into a specimen tube, and some of it drawn up into a standard Westergren tube (2.5 mm. in diameter) to the zero mark, 200 mm. from the tip. The tube is set vertical in a stand and the level of the red cell column read after one hour. In men the normal rate is 3 to 5 mm. at the end of one hour, and in women and children 4 to 7 mm. In

active infections these rates are much increased. A correction should be made where the degree of anæmia is severe.

Interpretation. The normal S.R. in one hour for males is 4 mm. and females 6 mm. Menstruation causes variation and tests should be avoided at these times. Pregnancy shows an increased rate, values over 20 being common after the fourth month. In *anæmia* there is some increase of S.R. governed by the number of cells, their size and their hæmoglobin content. S.R. finds its most important application in *tuberculosis*. Changes in S.R. are of importance because they are an accurate index of changes in the patient's state and of the effects of treatment. Thus it is said that patients after sanatorium treatment having a S.R. of more than 10 are not likely to do well. Finally, while a raised S.R. in a case of suspected early tuberculosis lends much support to the diagnosis it must be recognised that not infrequently early cases may show a normal S.R. In *anthracosis* and *emphysema* S.R. is normal. In simple *chronic bronchitis* it is normal or slightly raised; figures of 20 or more would suggest tuberculosis. In *acute rheumatism* the S.R. is usually above 20 and so long as it remains raised the disease is active even though physical signs have become normal. In *rheumatoid arthritis* very high figures—40 or more—are found. The raised S.R. may persist after the arthritis has subsided and is an important indication that the disease is still active. In *coronary thrombosis* the S.R. rises on the second or third day. In *appendicitis* the S.R. is usually raised on the third day, but it should not be considered a factor in diagnosis. S.R. is raised in *acute salpingitis* and it is said that this might help to distinguish the condition from tubal gestation. In most *acute infections* a great rise in S.R. soon occurs but in typhoid the rise is only slight for the first fortnight. In uncomplicated *influenza* S.R. is low—under 20 and often under 10. In *malignant disease* it is normal unless the condition is very advanced.

Finally the S.R. may be of value in the type of case where a patient has been rather unwell for some time and has a temperature in the neighbourhood of 99°. Here a raised S.R. is a special indication for close investigation—it is possible that some cryptic infection like perinephric abscess, endocarditis or early phthisis may come to light.—Montague Maizels, *Lancet*, ii/1936, 936.

From the standpoint of practicability and accuracy the sedimentation rate is the most useful laboratory test to aid in the diagnosis and evaluation of patients with active rheumatoid arthritis. A positive result was obtained in 92.2% of 154 tests carried out on 49 patients.—C. L. Short, L. Dienes and W. Bauer, *J. Amer. med. Ass.*, i/1937, 2087.

The addition of chlorocresol as a preservative to solution of sodium citrate, 3.8%, used in determining the blood sedimentation rate introduces errors into the results. Phenylmercuric nitrate, 0.002 per cent., however, does not have any effect and is recommended for this purpose.—H. Henriksen, *Pharm. J.*, i/1942, 14.

The sedimentation rate and the sedimentin index.—B. L. Della Vida, *Brit. med. J.*, i/1942, 278.

For sedimentation rate in leprosy, see p. 918.

Detection of Blood Stains

Blood Stains on Clothing, etc. Plunge the cloth into boiling water for a few minutes, place on slide and add a few drops of ammonium sulphide. Examine microspectroscopically for absorption bands of hæmochromogen. May be increased by 10% potassium cyanide solution. If on a weapon or piece of jewellery, moisten with ammonium sulphide, scrape off sufficient and examine as before.

Oxyhæmoglobin in solution with a little sodium chloride evaporated over sulphuric acid to syrupy consistence. Mixed with fifteen times its volume of glacial acetic acid and heated on a water-bath for several hours the mixture yields, on cooling, flat rhombic crystals of hæmatin hydrochloride with dark violet colour and lustre—this is one of the recognised tests for blood stains.

Guaiacum Test. The stain must give a red aqueous extract yielding no coloration to a straw-coloured solution of guaiacum in alcohol 90% when applied by itself, but a blue coloration within one second on further addition of hydrogen peroxide. Oxidisers and enzymes give a reaction with guaiacum solution *alone*. Blood does not.

Benzidine Test. using tablets of benzidine 0.1 g. and sodium perborate 0.1 g. Just before use, dissolve a tablet in 10 ml. of glacial acetic acid. If a suspected spot on an article of clothing, etc., is to be examined, it is moistened with a drop of normal saline and well rubbed with a glass rod. The drop is then absorbed in a small piece of absorbent cotton wool and the spot at once treated with a few drops of the reagent. In presence of blood a blue colour is seen. The benzidine test is very sensitive and simple.

Hæmochromogen Crystal Test. Reagent—Takayama's Solution 2 (an improvement on an earlier Solution 1, used in 1912). Caustic soda solution 10% 3 ml., pyridine 3 ml., saturated grape sugar solution 3 ml., distilled water 7 ml. The solution keeps for one to two months. Two or three drops are added to a small piece of the suspected material on a slide and covered with a cover glass. Salmon-pink crystals of characteristic appearance usually appear within 6 minutes in the cold. By warming the slide until bubbles just appear the crystals are formed almost at once. In the many types of stains tested the hæmochromogen crystals were readily obtained cold, whereas in some cases hæmin crystals were not obtained at all, or only with difficulty.

Kastle-Meyer Test. (Meyer's phenolphthalein reagent, as described under *Fæces*, is used.)

The technique consists in adding 10 to 20 drops of the reagent to the suspected blood-stained surface, then adding a few drops of fresh 20 vol. hydrogen peroxide. If blood is present, a deep permanganate colour develops almost immediately. The test is of value for detecting hæmoglobin.—J. Glaister, *Brit. med. J.*, i/1926, 650.

Doubt as to reliability of the test. Aspirin and other drugs, also a red meat diet, may confuse. Cannot be accepted in a court of law.—D. Kerr, *Brit. med. J.*, i/1926, 721.

Precipitin Test. Precipitins are formed when the serum of one kind of animal is introduced into the body of another species, e.g., the serum of a horse injected into a goat causes the serum of the goat to be capable of forming a precipitate with normal horse serum.

In using the test for forensic purposes a rabbit is injected with human blood serum. The serum of the rabbit, "anti-human serum," when dropped into a clear solution of human serum causes a precipitate—not with the serum from another animal. The principal difficulty in the test is to obtain from the rabbit an anti-human precipitating serum of the proper strength. To be thoroughly reliable and specific the formation of the precipitate must begin in five minutes and be complete in thirty minutes. Old blood-stains respond as well as recent.

A human blood-stain taken up with normal saline and some anti-human serum added gives a white cloudy ring—not so the stains from animal blood. Specific sera injected into a rabbit form an equally specific anti-serum—in other words, human anti-serum will infallibly detect human blood; a horse anti-serum will detect horse's blood, and so on. (*For further details see Hawk.*)

Luminescence Test. Glen and Pfannistiel (*J. prakt. Chem.*, 1936, 146, 129, 137) described the preparation of 3-amino-phthalic-acid-hydrazide-hydrochloride, and indicated that the alkaline solution, with the addition of hydrogen peroxide or sodium peroxide produced a typical chemical luminescence with hæmatin. This reaction is the basis of the test. One of two solutions may be used: A. 3-amino-phthalic-acid-hydrazide-hydrochloride 1 g., sodium peroxide 5 g., distilled water 1000 ml. B. 3-amino-phthalic-acid hydrazide-hydrochloride 1 g., sodium carbonate 50 g., hydrogen peroxide (10 vol.) 50 ml., distilled water 1000 ml. The solutions remain usable for years by the addition of 10% of 20-vol. hydrogen peroxide to reactivate the quantity of stock solution required for a particular test.

The solution is introduced into an all-glass pressure-spray to produce a fine mist from a watery solution. When the mist falls on a stain containing hæmatin the result is a marked bluish-white luminescence clearly visible in the dark, which persists for some minutes. The delicacy of the test in the presence of fresh blood containing little hæmatin may be enhanced by adding a few drops of NaOH solution (about 30%) to the suspect fluid or suspension in a test-tube, shaking the tube and adding the test solution. The reaction is specific and is positive with blood in dilutions over one in a million.—J. McGrath, *Brit. med. J.*, ii/1942, 156.

BLOOD ANALYSIS

Acetone

Rothera's test (*see* p. 637) can be applied directly to about 5 ml. of non-hæmolyzed serum or plasma and will detect a gross excess of the ketones. It is better, however, to precipitate the proteins first without hæmolysis; normal blood filtrates remain colourless, those containing a slight excess of ketones slowly turn a pale-purplish pink, and in marked ketonæmia a definite purple colour develops in a minute or less.

Colorimetric method for the determination of acetone bodies in blood, based on a reaction with salicylaldehyde.—*Yearb. Pharm.*, 1927, 93.

The acetone content of the blood is 43.5% higher in pregnant than in non-pregnant women. During the second stage of pregnancy it reaches 12 mg. per 100 ml. of blood, returning to normal shortly after delivery.—*per J. Amer. med. Ass.*, ii/1925, 861.

Bilirubin

Normal serum usually contains from 0.1 to 0.3 (never more than 0.5) mg. per 100 ml. Hyperbilirubinæmia is met with clinically in mechanical obstruction of the bile ducts, in accelerated blood destruction and in diffuse liver injury. There are numerous tests of faulty hepatic functioning based on the determination of hyperbilirubinæmia, and of these the most important is Van den Bergh's test.

LIVER FUNCTION TESTS

Van den Bergh Test. Bilirubin which has been excreted by the liver and reabsorbed appears to differ from that which has not passed through the liver cells, in that it gives a positive direct Van den Bergh reaction and is readily excreted by the kidney, whereas the latter, which is believed to be combined in some way with plasma protein (or present in the form of a colloid, instead of a crystalloid), gives only an indirect reaction and is not excreted in the urine unless the concentration in the plasma becomes very high. A "biphasic" reaction is explained by the simultaneous presence of both types of bilirubin. The qualitative Van den Bergh reaction, therefore, detects and differentiates between "free" (crystalloid) and "combined" (colloid) bilirubin and the quantitative Van den Bergh measures the total of bilirubin (of both types) present.

The reagents employed in the test are as follows:—Ehrlich's diazo reagent: *Solution I.*—Sulphanilic acid 1.0 g., conc. HCl 10 ml., water to 200 ml. *Solution II.*—Sodium nitrite 0.5 g. in 100 ml. of water. Immediately before use add 0.1 ml. of Solution II to 10 ml. of Solution I. *Standard Solution.*—Dissolve 2.161 g. of anhydrous cobalt sulphate in 100 ml. of water to which 0.5 ml. of conc. H_2SO_4 has been added. This keeps indefinitely in the dark and corresponds in colour to 0.5 mg. of bilirubin in 100 ml.

Direct Qualitative Test. In each of three tubes put 0.25 ml. of serum (free from hæmoglobin). To the first tube add 0.2 ml. of water, and to the second add 0.2 ml. of diazo reagent, shake, and wait 5 to 10 minutes. If any colour appears add 0.2 ml. of diazo reagent to the third tube and observe constantly, noting when the colour first appears and when it becomes maximal. If no colour appears in 5 minutes add 0.5 ml. of 95% alcohol, shake, and note whether any colour has appeared.

An immediate direct reaction is indicated by the prompt appearance of a purplish-red colour which becomes maximal within 30 seconds. A biphasic reaction is indicated by the appearance within 30 seconds (prompt biphasic) or 60 seconds (delayed biphasic) of the red colour, which gradually increases in intensity. A reaction which does not appear until more than a minute has elapsed has about the same significance as a positive indirect reaction. If no colour appears within one minute but does appear promptly after adding

alcohol this constitutes a positive indirect reaction. Any serum giving a positive direct reaction will also give a positive indirect reaction.

Indirect Quantitative Test. In a graduated centrifuge tube put 1 ml. of serum. Add 0.5 ml. of diazo reagent and shake. Add 2.5 ml. of 95% alcohol and shake. Add 1 ml. of saturated ammonium sulphate solution, mix, and allow to stand for 15 minutes. Centrifuge until clear. Compare with standard solution in a colorimeter or comparator.

Reading of standard \times 4 (dilution) \times 0.5 = mg. bilirubin per 100 ml.
Reading of unknown

Van den Bergh's "unit" corresponds to 0.5 mg. per 100 ml.

In obstructive jaundice a positive direct reaction is obtained. In hæmolytic anæmias an indirect reaction is usual. In diffuse disease of the liver any type of reaction may be obtained, depending on the stage and severity of the process. The reaction is, therefore, not of help in distinguishing jaundice due to mechanical obstruction of the ducts from other types. It is not a sensitive indicator of liver injury, since many cases occur without hyperbilirubinæmia.

Description of an improved technique for determination of bilirubin in the serum by means of the diazo method. The first improvement is the colorimetric determination in monochromatic light by means of a dimming wire gauze, instead of the fluid for comparison formerly used. This instrument is standardised with azo-bilirubin, derived from chemically pure bilirubin. The second improvement is the prevention of the absorption of bilirubin on the albuminous precipitate which occurred with the old technique. This result is achieved by adding in suitable proportions to the serum a mixture of reagent, diluted alcohol and a buffer.—A. A. H. van den Bergh and W. Grotepass, *Brit. med. J.*, i/1934, 1157.

Icterus Index (Meulengracht). The colour of the serum is compared with that of a standard 1 : 10,000 solution of potassium bichromate, the colour of which is taken as an index of 1. *Standard solution*:—potassium bichromate 0.1 g., conc. H_2SO_4 0.1 ml., water to 1000 ml.

The normal range lies between 1 and 4. Any increase is usually due to bilirubin. It is not so useful as the Van den Bergh test, but is of value in confirming a diagnosis of carotinæmia where a highly coloured serum does not show an increased bilirubin content by the Van den Bergh test. For the test the serum must be free from hæmoglobin and in the collection of the blood hæmolysis must be avoided.

Fouchet's Test. To 1 volume of serum add 1 volume of Fouchet's reagent (*vide p.* 645). A blue or green colour appears due to the oxidation of the bilirubin. Normal sera give negative results. Positive reactions are given by sera containing more than about 1.5 mg. of bilirubin. It is useful as a quick spot test for the presence of increased bilirubin in the blood. It will not distinguish between obstructive and hæmolytic jaundice. The test is best carried out by adding a drop of serum to a drop of reagent on a white porcelain tile.

Phenoltetrachlorophthalein Test (Rosenthal). 5 mg. of the dye are injected intravenously for each kg. bodyweight. 5 ml. of blood is taken from the other arm exactly fifteen minutes after the injection and again one hour after the injection. The blood is allowed to clot and the sera or plasma separated, hæmolysis being avoided. A trace of alkali is added to each of the sera to develop the maximum colour and the amount of dye present in each serum is determined colorimetrically by comparison with standard tubes of the dye (*see* Bromsulphthalein Test). Results are expressed as percentages of an assumed initial concentration of the dye in the plasma.

Normally, only from 2 to 6% of dye remains in the circulation after 15 minutes and less than 3% at the end of an hour, but in hepatic disorders there is an appreciable amount (up to 50%) of the dye still in circulation at the end of an hour.

Bromsulphthalein Test. (Phenoltetrabromphthalein sodium sulphonate). 2 mg. of the dye are injected intravenously into the arm for each kg. bodyweight, a 5% solution being employed and the injection made slowly and carefully, avoiding leakage. After half an hour 5 ml. of blood is withdrawn from the opposite arm, avoiding hæmolysis. The serum is separated and 1 ml. placed in each of two comparator tubes. To one of the tubes add 1 or 2 drops of 5% NaOH and to the other 1 drop of 10% HCl. The colour of the unknown is then compared with that of the standard in a comparator, backing the

standard tube with the tube containing acidified serum and the one containing alkalinised serum with a tube of distilled water. The 100% standard is prepared by dissolving 4 mg. (0.08 ml. of 5% solution) of the dye in about 70 ml. of water, adding 5 ml. of 0.5% NaOH and making up to 100 ml. with water. A series of standards from 5% upwards is prepared by dilution with 0.025% NaOH.

Normally, half an hour after the injection, all the dye has disappeared and the presence of 10% or more of the dye after that period indicates impaired liver function. There is no close correlation between the amount of dye retained and the gravity of the disease.

Rose Bengal Test. Rose Bengal can be either the potassium salt of tetraiodo-di- (or tetra-) chlorofluorescein, or of hydroxytetraiodo-di- (or tetra-) o-carboxy-phenylfluorone, or the sodium salt of the two dichloro compounds. It can be obtained by the action of iodine on dichlorofluorescein in the presence of potassium chlorate and cupric chloride (dichloro compound), or by acting on tetrachlorofluorescein with iodine to produce the tetrachloro body. It is a dark or brownish-red powder soluble in water without fluorescence.

Withdraw sample of blood from vein in the cubital fossa and discharge into graduated centrifuge tube containing 2 ml. of 2% potassium oxalate solution. Without removing needle from vein inject 100 mg. of dye (150 mg. in large persons) in sterile 1% salt solution. Leave needle in vein and 2 minutes after injection withdraw 10 ml. of blood from needle (still *in situ*) into a fresh syringe and discharge into another centrifuge tube containing 2 ml. of oxalate solution: repeat this at 4 and 8 minutes after dye injection. Withdraw needle and leave patient in darkened room for an hour. Centrifuge blood samples at 2000 revs. for $\frac{1}{2}$ hour, and note percentage of cells and plasma in each tube. From the last three samples dilute 3 ml. of plasma in separate tubes with an equal volume of salt solution and compare the colours in a Hellige colorimeter with standard solution containing 5 ml. plasma from first tube and 5 ml. of 0.0075% rose bengal solution. The colorimeter reading is corrected to allow for the 2 ml. oxalate solution. Having obtained the concentration of the dye in the 2-minute sample, and knowing the total amount of dye injected, calculate the blood volume of the person. For the purposes of comparison, a standard blood volume of 7000 ml. is taken, and the final concentration is obtained by multiplying the corrected reading by the blood volume and dividing by 7000.

The dye is eliminated almost entirely from the blood stream through the liver. It remains in the circulation for a sufficient length of time for determination of the dye in the plasma to be made. Patients with definite cirrhosis or other extensive liver disease show marked retention of the dyes in the blood.

Lævulose (Fructose) Tolerance Test. All ordinary sugars, dextrose, cane sugar, etc., when ingested, raise the concentration of sugar in the blood, but lævulose does not. Thus, with 50 g. of dextrose the blood sugar concentration is increased in the first half hour from the usual 0.1 to 0.16 or 0.17%, returning to normal in about 1½ hours. There is no rise with lævulose. This lack of power on the part of lævulose depends on an intact liver. If there is a definite lesion of the liver, lævulose acts, more or less, like dextrose, i.e., there is a marked increase in blood sugar.

To conduct the test, obtain blood from the fasting patient for a sugar determination. Give 50 g. of pure lævulose in 250 ml. of water, take samples of blood at half-hourly intervals for two hours and make sugar determinations on each. The following figures are indicative of impaired tolerance (Stitt): (1) a maximum over 130 mg. (providing the fasting level is not over 115 mg.); (2) a rise of not less than 30 mg. if the fasting sugar is from 80 to 100 mg. (or from 35 to 40 mg. if the fasting level is less than 80); (3) failure to return to within 15 mg. of the fasting level after two hours. The test is not of value in detecting slight degrees of injury and is not applicable to diabetics.

Determination of lævulose in blood. A more satisfactory test can be made on the basis of determining the actual lævulose in the blood than by taking account of the total blood sugar values.

To 1 part of blood add 7 parts of distilled water. Allow a few minutes for hæmolysis, then add 1 part of 10% $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and 1 part of 0.5% NaOH. Place 2 ml. of the filtrate in a test-tube. Place in each of 3 similar test-tubes 2 ml. of standard fructose solution, the standards used containing 0.1, 0.05, and 0.025 mg. of fructose per ml. To each of the tubes add 2 ml. of 0.1% alcoholic resorcinol solution and 6 ml. of 30% HCl. Shake the tubes vigorously and place in a water-bath adjusted to maintain a temperature of 80°. Keep in

the water-bath for 8 minutes. Remove the tubes to a beaker and cool with running water. Compare in a colorimeter in the usual way, the standard most closely matching the unknown being used.—J. H. Roe, *J. biol. Chem.*, 1934, 107, 15.

A method for the estimation of fructose in the blood based on the reaction between bile salts and fructose. The delicacy of the reaction is such that fructose contents of the order of 5 to 20 mg. per 100 ml. can be estimated.—L. D. Scott, *Biochem. J.*, 1935, 29, 1012.

Take 2 ml. plasma in a 15 ml. centrifuge tube. Add 2 ml. of water and 2 ml. of zinc sulphate solution (10% $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$). Close the tube with a rubber stopper and shake. Add 2 ml. caustic soda solution (0.5 N. NaOH) and again shake. Centrifuge well and filter any supernatant liquid through a small filter paper. In a pyrex test-tube, graduated at 10 ml., take 2 ml. of filtrate and 6 ml. acid-alcohol-diphenylamine reagent (ethyl alcohol, 70 parts; concentrated HCl, 50 parts; 20% diphenylamine in alcohol, 6 parts). Place the tube in a boiling water-bath for exactly 15 minutes, and cool rapidly in cold water. Dilute to 10 ml. with alcohol and compare in a colorimeter with standard solutions of fructose similarly treated. (Standard fructose solution: 1% fructose in saturated benzoic acid diluted as required to contain 0.025 or 0.1 mg. fructose in 2 ml.)—F. K. Herbert, *Biochem. J.*, 1938, 32, 815.

A method utilising the blue colour formed by treatment with diphenylamine and hydrochloric acid to measure the amount of fructose present, *n*-propyl alcohol being employed to produce a clear solution. The protein is removed by means of cadmium hydroxide and the resulting solution can be used for the estimation of both fructose and glucose. Only 1 ml. of blood is required and the error involved is about 5%.—R. W. Martin, per *Analyst*, 1939, 692.

Intravenous Galactose Tolerance Test. A solution of galactose (50 g. per 100 ml. of solution) is prepared and sterilised by filtration and steaming. The patient receives no breakfast. 50 ml. of the 50% solution of galactose is injected intravenously, the injection taking about 5 minutes. The first sample of blood is taken immediately before the administration of the galactose, the second sample at $\frac{1}{2}$ hour, and further samples at 1, $1\frac{1}{2}$ and 2 hours. The samples of blood (0.2 ml. of capillary blood) are washed into centrifuge tubes containing 2.2 ml. of isotonic sodium sulphate; 1 ml. of yeast suspension is added. (Fresh baker's yeast is shaken with five times its weight of distilled water, 10 ml. of the mixture transferred to a centrifuge tube, and the yeast centrifuged down. The yeast is washed three times by stirring up with 10 ml. of fresh distilled water and centrifuging. It is finally stirred and shaken with 10 ml. of isotonic sodium sulphate.) The contents of the tube are thoroughly mixed and are kept at 37° for 15 minutes, which completely removes the glucose. Then 0.3 ml. each of a 10% solution of sodium tungstate and of a 7% solution of copper sulphate is added and the mixture gently shaken. The precipitated proteins and yeast are centrifuged or filtered and 2 ml. of the filtrate (equivalent to 0.1 ml. of blood) is used for the estimation of the sugar. If the Harding copper method is employed the factor 162 is used for conversion of the titration figure (ml. of 0.005 N thiosulphate) into mg. of galactose per 100 ml. of blood. The galactose values are plotted against time and for a normal person usually give a curve which begins about 200 mg. of galactose per 100 ml. of blood, falls steeply during the first hour and reaches a figure between 0 and 10 mg. per 100 ml. at the end of two hours. In most cases of obstructive jaundice the curve of blood galactose follows the same course, there being little or no galactose left in the blood at the end of two hours. In conditions of liver damage the level of galactose in the blood does not return to the normal level within the two hours. The test will not detect minor degrees of liver damage in patients who are not jaundiced.—E. J. King and R. S. Aitken, *Lancet*, ii/1940, 542. (See *Lancet*, i/1937, 886, for description of Harding Copper Method.)

For a description of the Galactose Tolerance Test in Urine see p. 654.

Quick's Hippuric Acid Test of liver function. See Urine Analysis this volume, p. 644.

Comparison of Liver Function Tests. For practical purposes it may be said that: (1) in types of disease of the liver not associated with jaundice, information gained from the study of retention of bromsulphthalein is as reliable as any, and under these conditions other tests give chiefly confirmatory evidence; (2) in cases of jaundice, some information, which is not altogether reliable, as to the possible hepatogenous or obstructive nature of the jaundice in any case can be had by studies on excretion of galactose, the value for cholesterol and

cholesterol esters in the plasma, and the value for serum phosphatase; and (3) the best information as to the state of functional activity in the liver in cases of jaundice can be gained from a consideration of the value for serum bilirubin, its daily variations, and a knowledge of the anatomic changes which these may represent. So far as indirect methods of measuring liver function in the presence of icterus are concerned the hippuric acid test gives reasonably accurate results, which should not, however, supplant the impressions gained from purely clinical study. (A clinical review of liver function tests in use at the Mayo Clinic.)—A. M. Snell and T. G. Magath, *J. Amer. med. Ass.*, i/1938, 167.

Calcium

Serum or citrated plasma normally contains 9–11 mg. of total calcium per 100 ml. Of this calcium, part is diffusible and part is non-diffusible. Furthermore, a portion is ionised and the rest is non-ionised. The chief value of calcium estimations is in the diagnosis of tetany, particularly of infantile tetany. In different diseases the serum calcium may be normal, lowered or raised. High serum calcium figures are found in cases of parathyroid tumours (generalised osteitis fibrosa), after the administration of an excess of parathyroid extract, and in some cases of multiple myelomatosis. Low serum calcium figures are found in tetany due to deficient parathyroid secretion and in the infantile (rachitic) type of tetany. Also in chronic azotæmic nephritis (advanced cases), renal infantilism (some cases), rickets (the minority of cases), osteomalacia (some cases), coeliac disease (few cases), sprue (few cases), ideopathic steatorrhœa and chronic sepsis (a few cases).

Kramer and Tisdall's Method. The calcium is precipitated from serum by means of ammonium oxalate. The precipitate is centrifuged and washed with weak ammonia and after solution in dilute sulphuric acid the calcium oxalate is titrated with N/100 KMnO_4 .—*J. biol. Chem.*, 1921, 47, 475, and 1923, 56, 439.

Trevelan and Bainbridge's Method. 2 ml. of ammonium oxalate solution (3.5%) is measured into a centrifuge tube, having a steep conical end, 1 ml. of serum is added and the contents stirred vigorously. Allow the tube to stand 2 to 3 hours and then centrifuge at 3,000 revs. per minute for 10 minutes. Pour off supernatant fluid and drain tube over filter paper. Add a further 2 ml. of ammonium oxalate solution, centrifuge, pour off, and repeat with a fresh 2 ml. as before. Dry the precipitate by heating the centrifuge tube in the steam oven and convert the calcium oxalate into carbonate by passing the tube through a bunsen flame for one minute, any ammonium oxalate left being converted into ammonium carbonate and volatilised. After cooling, 1 ml. of N/100 phosphoric acid is added and, when solution is complete, one drop (0.016 ml.) of 0.04% bromophenol blue is added and titration carried out with N/50 sodium hydroxide, using a micro-burette or micrometer syringe. The end-point corresponds with a colour intermediary between those of buffer solutions of pH 4.0 and 4.2, and the difference between the titration figure so obtained, and that for the acid alone gives the amount of calcium in the serum taken. 1 ml. only of the serum need be taken, and results within 5% of the correct value have been obtained with 0.01 mg. of calcium per ml.

1 ml. of N/100 phosphoric acid is equivalent to 0.64 mg. of calcium oxalate or 0.2 mg. of calcium. Thus, 0.1 mg. of calcium which is a little more than the average content per ml. of serum, would combine with 0.5 ml. of N/100 phosphoric acid.—*Biochem J.*, 1926, 423–426.

Schwartz Method. The following method will find easy application where only occasional calcium determinations are made and facilities are inadequate.

Place in a conical centrifuge tube 0.5 ml. of ammonium oxalate solution and 2 ml. of citrated plasma. Then add another 0.5 ml. of ammonium oxalate solution and make up to 6 ml. Mix thoroughly with distilled water and let stand for one-half hour. Allow the precipitate to form in the ice box overnight. Centrifuge at 1500 to 1800 r.p.m. until sedimentation is complete and

carefully pipette off the supernatant fluid, leaving 0.3 ml. in the tube. The precipitate is retained undisturbed. Repeat the washing *not less than three times*, using about 3 to 4 ml. of ammonia water, thoroughly stirring the precipitate by rotary motion of the tube. Use no stopper. After each washing centrifuging should be continued until the precipitate is firmly packed in the conical tube end. Last, dissolve the precipitate in 2 ml. of N/1 sulphuric acid by placing in a water-bath and heating to 75°. Simultaneously, employ 2 ml. of calcium chloride solution and treat exactly the same as the serum. Titrate with potassium permanganate solution made for the purpose. It is best to titrate the standard calcium chloride solution first to a faint pink and then match this tint with that obtained in titrating the calcium serum.

Calculation:

$$\frac{C \times S}{P} \times 50 = \text{serum calcium.}$$

C = mg. of calcium in 2 ml. of standard calcium chloride solution (0.2 mg.).

S = ml. of KMnO_4 used in titrating the serum.

P = ml. of KMnO_4 used in titrating the standard calcium chloride solution.

Solutions required.—1. *Standard calcium chloride solution:* Dissolve 0.2498 g. of pure calcite (calcium carbonate) in a little dilute HCl in a wide evaporating dish of 50 to 100 ml. capacity, care being taken to avoid loss by spattering. Carefully evaporate the solution several times to near dryness, each time adding distilled water, and last, evaporate to near dryness, expelling the last traces of HCl. Dissolve the residue in distilled water and dilute to 1 litre; 1 ml. of this solution equals 0.1 mg. of calcium. 2. *Potassium permanganate solution:* Dissolve about 0.030 to 0.040 g. of potassium permanganate crystals in 100 ml. of distilled water. Keep in dark bottle. The solution need not be standardised. 3. *Ammonium oxalate solution* 3%. 4. *Approximately N/1 sulphuric acid:* 28 ml. H_2SO_4 added to 970 ml. of distilled water. 5. *Ammonia water* 2%: If sodium citrate is used as anticoagulant, correction for the dilution should be made when final calculation is completed.—I. Schwartz, *J. Lab. clin. Med.*, 1936, 27, 426.

Chlorides

Whole blood normally contains from 450 to 530 mg. of sodium chloride per 100 ml. and plasma from 560 to 620 mg. Higher values are obtained in certain cardiac diseases, eclampsia, prostatic obstruction, and anæmia; lower values may occur in diabetes, uræmia, persistent diarrhoea or vomiting, intestinal obstruction, severe burns, fevers, and pneumonia; the results in nephritis are variable. The chief value of chloride determination is to detect and measure chloride depletion as a guide to treatment with sodium chloride. For the accurate determination of plasma chlorides it is important that the blood should be collected under oil, since there is a shift of chlorides from the cells to the plasma when blood is exposed to the air owing to the escape of CO_2 .

Whitehorn's Method. To 10 ml. of Folin-Wu tungstic acid filtrate (see p. 696) add 5 ml. of standard silver nitrate solution (containing 4.791 g. per litre, 1 ml. = 1 mg. Cl), stir, add 5 ml. of conc. nitric acid, and allow to stand for 5 minutes. Add 0.3 g. of ferric ammonium sulphate and titrate the excess of silver nitrate with standard thiocyanate solution until a salmon-red colour persists for 15 seconds in spite of vigorous stirring. (The standard thiocyanate solution is prepared by dissolving 2.5 g. of ammonium thiocyanate in a litre of water; titrate against standard silver nitrate solution and dilute so that 5 ml. are equivalent to 5 ml. of the silver nitrate solution.)

Calculation:

$$(5 - \text{No. of ml. of thiocyanate solution}) \times 100 = \text{mg. Cl per 100 ml. of whole blood.}$$

Rose's Method. For blood serum or plasma 1 ml. of the fluid is mixed with 3 ml. of water, 3 ml. of 0.2N sodium acetate solution, and 3 ml. of 0.2N acetic acid, heated in a boiling water-bath for 2 minutes, cooled and filtered; 7.5 ml. of the filtrate and 3 ml. of 0.2N sodium acetate solution are diluted with 10 ml.

of water, 2 ml. of dichlorofluorescein solution is added and the whole titrated in the usual way. In the presence of much urea, or if less than 7.5 ml. of filtrate can be obtained, it is advisable to add 1 ml. of 1% sodium chloride solution before titration, and then to perform a blank using 10 ml. of buffer solution of pH 5, 10 ml. of distilled water, 1 ml. of 1% sodium chloride solution and 2 ml. of indicator solution. For whole blood, 9 ml. of buffer solution of pH 5.3 and 1 ml. of blood are heated in a boiling water-bath for 2 minutes, cooled and filtered, and to an aliquot part of the filtrate is added 0.3 ml. of 0.2N acetic acid for each 2.5 ml. of filtrate, 1 ml. of 1% solution of sodium chloride and 10 ml. of water, and the whole is then titrated as before using dichlorofluorescein as indicator, a blank being also performed.—*Biochem. J.*, 1936, 1140.

Cholesterol

The normal range of total cholesterol is 100–200 mg. per 100 ml. of whole blood and 100–200 mg. per 100 ml. of plasma or serum. Hypercholesterolaemia is met with in xanthomatosis and in nephrosis; in the latter, the figure is frequently between 300 and 800 mg. per 100 ml. The finding of hypocholesterolaemia is of little or no value clinically. Cholesterol estimations are not of much value in the diagnosis of gall-stones.

High values, such as 300 mg. per 100 ml. or over, are found in pregnancy, myxoedema and the so-called "nephrotic syndrome." Cholesterol may be increased in diabetes, but this is not invariable. After a meal there is usually a slight rise in total cholesterol, and after the ingestion of meat this rise may be prolonged. In liver disease, especially when icterus is present, cholesterol is elevated. The ratio of esters to free cholesterol is characteristically low in parenchymatous disease of the liver.—W. T. Salter, *New Engl. J. Med.*, i/1941, 22.

Hypercholesterolaemia possessing the "nephrotic" syndrome, marked by profuse parenchymatous degeneration, heavy albuminuria, changes in the plasma protein, and with oedema as the chief symptom. It is not found in interstitial nephritis, arteriosclerotic kidney, or arteriosclerosis. In myxoedema the total cholesterol of plasma may be increased by nearly 50% above normal. Cholesterol metabolism in disease.—J. A. Gardner, *Brit. med. J.*, ii/1932, 392.

Obermer and Milton's Method. About 12 ml. of a 3:1 alcohol-ether mixture is placed in a 20 ml. graduated flask and 1 ml. of plasma is delivered into the mixture from a very fine pipette. The flask is well shaken and then heated on a boiling water-bath until the contents begin to boil, it is then again vigorously shaken and boiling continued for 3 minutes after which it is allowed to cool to room temperature and the liquid made up to 20 ml. with the alcohol-ether mixture. **Free Cholesterol.** 10 ml. of the solution is evaporated to dryness with 0.5 ml. of 1% digitonin solution (prepared by dissolving 1 g. in 50 ml. of dehydrated alcohol, adding 50 ml. of water, allowing to stand in ice overnight and filtering if necessary), the last traces of alcohol being removed on the water-bath. 2 ml. of water is added to the residue, the liquid boiled, 4 ml. of acetone added and the liquid transferred to a centrifuge tube where 1 drop of 4% aluminium chloride solution followed by 1 drop of concentrated ammonia solution is added. After centrifuging at a high speed for 1 minute the liquid is poured off and the precipitate dissolved in 1 drop of 30% HCl. The original precipitation vessel is rinsed with a further 3 ml. of acetone, the washings added to the centrifuge tube and the whole again centrifuged for 5 minutes. The liquid is again decanted, the precipitate washed with 2 ml. of acetone, followed by 3 ml. of ether, the ethereal liquid being centrifuged, decanted and the residual ether allowed to evaporate spontaneously. 3 ml. of a 3.5% solution of anhydrous zinc chloride in glacial acetic acid is added and then 2 ml. of 10% o-nitrobenzoyl chloride in glacial acetic acid, freshly prepared. The tube is covered and heated in a boiling water-bath for 50 minutes. The colour produced is read in a suitable photometer previously standardised against known quantities of cholesterol. **Total Cholesterol.** 5 ml. of the alcohol-ether extract is boiled under a reflux condenser for 30 minutes with 0.2 ml. of 20% sodium ethoxide in alcohol. The bulk of the alcohol is boiled off and 15 ml. of light petroleum added. The liquid is boiled again, shaken well, 2 ml. of water added and again shaken. After settling the light petroleum layer is poured off through a filter into a dry

flask and the residue washed twice with 10 ml. of light petroleum. To the combined filtrates 0.5 ml. of digitonin solution is added, the solvent distilled off, 10 ml. of ether-alcohol added and the contents of the flask evaporated to dryness. Further procedure is as described above.—*Biochem. J.*, 1933, 345. Further details of the above process.—*J. Lab. clin. Med.*, 1937, 22, 943.

Liebermann-Burchard Reaction. Mix 6 ml. of alcohol (95%) and 2 ml. of ether in a dry centrifuge tube, add 0.1 ml. of whole blood, cork, shake vigorously for 1 minute and then let the tube lie semi-horizontally with an even distribution of the sediment for 30 minutes. Centrifuge for 3 minutes and decant the liquid into a standard Evelyn photo-electric colorimeter tube. Evaporate to dryness in an incubator at 37°, inclining the tube at 15° and using a current of warm, dry air to hasten the process. Add 10 ml. of anhydrous chloroform, 4 ml. of acetic anhydride, and 0.1 ml. of concentrated sulphuric acid, and keep in a refrigerator at -5° to +5° for 45 minutes. Then determine the intensity of the colour by the Evelyn colorimeter using a filter transmitting light of wavelength 6300μ to 6600μ.—*M. Pijoan and C. W. Walter, J. Lab. clin. Med.*, 1937, 22, 968.

Phosphorus

The types of phosphorus compounds found in whole blood have been classified as: (1) Acid-soluble phosphorus (including inorganic phosphate and ester, or "organic phosphorus," compounds): (2) Lipin phosphorus (phosphatides): (3) Residual phosphorus. Of these fractions the only one whose determination is of direct clinical value is inorganic phosphate, and estimations are usually made on plasma separated as soon as possible after withdrawal of the blood. The normal range for adult's serum or plasma, lies between 2 and 4 mg. per 100 ml., and that of healthy children, between 4 and 6 mg. per 100 ml. Low values are found in rickets, and the estimation is of great value in early diagnosis. Insulin and adrenaline also cause a fall and there is a slight fall during pregnancy and in hyperparathyroidism. High values are found in hypoparathyroidism, in diabetic coma, in some cases of renal infantilism and during delayed healing of major fractures. In chronic nephritis, inorganic phosphate retention has important prognostic significance.

In 60 normal healthy adults between the ages of 20 and 40 years the mean serum calcium was 10.4 mg., the inorganic phosphorus 3.8 mg., and the phosphatase 7.2 units (King and Armstrong). In 15 healthy people aged 60 to 78 the serum calcium and inorganic phosphorus were not found to be significantly different from those of younger adults. The serum phosphatase was slightly raised in these older people.—*J. D. Robertson, Lancet*, ii/1941, 87.

Brigg's Method for Determination of Inorganic Phosphate. Place in a test-tube 2 ml. of plasma, 6 ml. of distilled water, 2 ml. of 20% trichloroacetic acid; shake for a few seconds, allow to stand for 10 minutes and filter through a double-acid-washed filter paper. In a glass-stoppered test-tube graduated at 10 ml. place 5 ml. of the protein-free filtrate, 2 ml. of molybdic acid solution, 1 ml. of sodium sulphite solution and 1 ml. of hydroquinone solution and add water to 10 ml. In a similar tube place 2 ml. of the phosphate standard, 2 ml. of molybdic acid solution, 1 ml. of sodium sulphite solution and 1 ml. of hydroquinone solution and add water to 10 ml. Stopper both tubes, mix by inversion, allow to stand for 30 minutes and compare in the colorimeter.

Calculation:

$$P = \frac{S}{U} \times 4.0 \text{ mg. per 100 ml. of plasma.}$$

The reagents used are: (1) *Molybdic Acid Solution*: Dissolve 25 g. of ammonium molybdate in 300 ml. of distilled water. Add 75 ml. of sulphuric acid to 100 ml. of distilled water. Cool, add to the ammonium molybdate solution and make up to 500 ml. (2) *Hydroquinone Solution*: Dissolve 0.5 g.

of hydroquinone in distilled water and make up to 100 ml., adding 1 drop of conc. sulphuric acid. (3) *Sodium Sulphite Solution*: Dissolve 20 g. of crystalline sodium sulphite in distilled water to 100 ml. and stopper well. (4) *Standard Phosphate Solution*: Dissolve 0.0878 g. of dry acid potassium phosphate (KH_2PO_4) in distilled water to 1000 ml. and add chloroform as preservative (2 ml. = 0.04 mg. P). (5) *20% Trichloroacetic Acid*.

A micro-chemical method for determination of plasma phosphatase.—E. J. King *et al.*, *Lancet*, i/1942, 207.

Potassium

Blood serum normally contains 18 to 21 mg. of potassium per 100 ml. The figure is raised in Addison's disease and lowered in familial periodic paralysis and in response to insulin injections. Whole blood normally contains from 150 to 250 mg. per 100 ml.

Jacobs and Hoffman's Method. The process depends upon precipitation of the potassium as a cobalt complex and determination of the cobalt colorimetrically with choline hydrochloride and sodium ferro cyanide. The potassium is precipitated by a sodium cobaltinitrite reagent (Kramer and Tisdall) prepared as follows. *Solution A*: 25 g. of cobaltous nitrate crystals dissolved in 50 ml. of water and 12.5 ml. of glacial acetic acid added. *Solution B*: 120 g. of sodium nitrite (potassium free) dissolved in 180 ml. of water. Add 210 ml. of solution B to all of solution A and when effervescence ceases draw air through the mixture to remove nitric oxide. Store in the ice chest and filter before use. The reagent will keep for at least a month.

1 ml. of serum is treated with 2 ml. of reagent in a 15 ml. centrifuge tube marked at 6 ml. After 45 minutes 2 ml. of water is added, the contents mixed and the tube centrifuged at a moderate speed for 15 minutes. The tube is then briefly inverted to drain, 1 ml. of water is added and the tube centrifuged for 5 minutes and again drained. In the same way the precipitate is washed twice with 2 ml. of alcohol (70%). After draining off the alcohol the precipitate is suspended in 2 ml. of water and dissolved by heating in a boiling water-bath for 10 minutes. After cooling, 1 ml. of 1% choline hydrochloride and 1 ml. of sodium ferrocyanide solution (2% $\text{Na}_4\text{Fe}(\text{CN})_6 \cdot 10\text{H}_2\text{O}$) are added and then water to 6 ml. The colour produced is compared with that produced by similarly treating a standard potassium sulphate solution containing 0.4011 g. of K_2SO_4 per litre (= 18 mg. of K per 100 ml.). Cobalt nitrate or sulphate solution previously standardised against potassium may also be used as a standard of comparison. The method is applicable also to the determination of potassium in urine and inorganic compounds.

A micro-chemical method for determination of serum potassium.—E. J. King *et al.*, *Lancet*, i/1942, 207.

Proteins

The proteins of plasma are albumin, globulin, and fibrinogen. The normal total figure of these is 5.8 to 8.6 g. per 100 ml. In serum the total proteins comprise albumin and globulin only, and amount to 5.6 to 8.5 g. per 100 ml. Low values for total protein are encountered in parenchymatous Bright's disease, in malnutrition, and in infections. High values are commonly found in vomiting and diarrhoea, e.g., in cholera, in multiple myeloma, and in lymphogranuloma inguinale. The globulin fraction tends to be high in hepatic inflammation but usually falls after extensive liver damage. Kala-azar commonly produces high globulin values.

Walther's Method. Wash out a Pyrex boiling tube (170 mm. \times 28 mm.) with distilled water. After washing out, about 0.5 ml. of water collects in it. Using a haemoglobin pipette, wash out 20 ml. of serum or plasma into this water. Add 0.25 ml. of pure sulphuric acid and mix. Boil gently over by-pass flame of Bunsen with an "anti-bump" rod (consisting of 5 mm. of glass tubing fused to the end of a glass rod 160 mm. long) in position. When thick white fumes rise and fill the tube, cover with a watch-glass and boil for three minutes. The

solution is then quite black. Remove the flame for two minutes and then add 0.5 ml. of saturated potassium persulphate solution. Mix and replace flame. Boil without watch-glass until thick white fumes rise again, then replace the watch-glass and boil again for a further two minutes, after which the solution will be clear and colourless. Cool, dilute with distilled water to the 25 ml. mark holding the tube with the 25 ml. mark at eye-level and the anti-bump rod removed. Replace the rod and wash well in the solution. Use any convenient volume and add 2 ml. of Nessler's solution. A similar volume of standard solution (ammonium sulphate, 0.03776 g. per litre of distilled water containing 7 ml. of pure sulphuric acid) is treated in the same way. Mix by inverting twice and compare at once in the colorimeter. Equal colour indicates 1000 mg. of total nitrogen per 100 ml. of sample. A direct reading may be obtained by fixing the unknown at "10" and moving the standard to match. The reading of standard $\times 100$ = mg. of total nitrogen per 100 ml. of sample. The reading is greatly facilitated by placing a spectrum blue colour filter over the eyepiece. Unless gross nitrogen retention is suspected a subtraction of 50 mg. to cover the non-protein nitrogen gives the figure for protein nitrogen which, when multiplied by 6.25/1000 gives the protein percentage. If a colorimeter is not available a comparator or similar instrument may be used, but the reading of the yellow Nessler colour is greatly helped by using a blue light filter.—W. W. Walther, *Lancet*, ii/1941, 337.

A micro method is proposed for the determination of blood proteins (total, globulin, serum albumin and fibrinogen) based on fractionation of the proteins with sodium sulphite and Kjeldahl digestion catalysed with copper selenite as recommended by Campbell and Hanna, and on direct nesslerisation of the diluted digest with the Nessler-Winkler reagent. Its rapidity and cheapness render it suitable for clinical use.—B. A. Mácola and C. Fazio, *Analyst*, 1941, 448.

A micro-chemical method for determination of plasma proteins.—E. J. King *et al.*, *Lancet*, i/1942, 207.

Estimation of plasma proteins by the salicylsulphonic acid reaction.—C. A. Mawson, *Biochem. J.*, 1942, 36, 273.

Sodium

Practically the whole of the sodium of the blood is in the plasma. The normal range for serum or plasma is 325–350 mg. per 100 ml. It is lowered in Addison's disease and its fall is usually accompanied by a rise in the serum potassium. The estimation is of use in the diagnosis of Addison's disease and also in following the effect of treatment with sodium chloride and extract of suprarenal cortex.

Modification of McCance and Shipp's Method. Mix in a centrifuge tube: serum, 1 ml.; water, 3 ml.; 20% trichloroacetic acid 1 ml.; centrifuge. In another centrifuge tube mix supernatant fluid 1 ml. (= 0.2 ml. of serum) and alcoholic zinc uranyl solution 10 ml. Stir with a thin glass rod until a precipitate of sodium zinc uranyl acetate forms. Stand at 0° for one hour. Centrifuge and drain off supernatant fluid by complete inversion of the tube. Wash precipitate with 5 ml. of 95% alcohol saturated with "triple acetate." Again centrifuge and drain. Add about 3 ml. of distilled water and shake until solution is complete. Transfer to 250 ml. flask with water. Add 1 drop of glacial acetic acid, 0.5 ml. of 20% potassium ferrocyanide and make up to 250 ml. mark with water. Mix and allow to stand for 3 minutes. Compare in a colorimeter with 1 ml. NaCl standard solution which has been treated in parallel with 10 ml. of alcoholic zinc uranyl solution and subsequently as the unknown.

If S = standard and U = unknown

$$\text{Then } \frac{S}{U} \times 0.7 \times \frac{100}{0.2} = \text{mg. of Na per 100 ml. of serum.}$$

The reagents used are: (1) *Alcoholic Zinc Uranyl Solution saturated with "triple acetate."* Dissolve 50 g. of uranyl acetate in 250 ml. of boiling 4% v/v acetic acid. Dissolve 150 g. of zinc acetate in 250 ml. of boiling 2% v/v acetic acid. Mix the two solutions whilst boiling. Bring to the boil again. Stand for 24 hours and filter if necessary. To the filtrate add an equal volume of absolute

alcohol. Stand for 48 hours at 0°, filter and store at 0°. Before use the reagent should be filtered or centrifuged to remove any suspended sodium zinc uranyl acetate. (2) 95% *Alcohol saturated with "triple acetate."* Add 50 ml. of (1) to 100 ml. of saturated solution of NaCl in 50% alcohol; allow to stand. Decant most of the supernatant fluid, centrifuge and wash the yellow precipitate thoroughly with 95% alcohol; suspend the precipitate in 95% alcohol, transfer to a glass-stoppered bottle, with more alcohol, and make up volume to about 500 ml. Shake thoroughly and store at 0°. There must always be undissolved precipitate at the bottom of the bottle. Centrifuge or filter enough for test when required. (3) *Stock NaCl* 0.89% = 0.35% Na. (4) *Standard NaCl* = 0.07% Na. Made by diluting 5 ml. of stock with water up to 25 ml. (1 ml. = 0.7 mg. of Na).

The alcoholic zinc uranyl acetate solution and the 95% alcohol, saturated with "triple acetate" should both stand for some time in melting ice before use. The precipitate of sodium zinc uranyl acetate may be estimated gravimetrically.

Butler and Tuthill's Method. This is a gravimetric determination in which the serum is first digested with acid to destroy the organic matter, the sodium then being precipitated as "triple acetate" and the precipitate filtered, washed, dried and weighed.

A micro-chemical method for determination of plasma sodium.—E. J. King *et al.*, *Lancet*, i/1942, 207.

Sugar

Generally speaking, in health, the amount of blood sugar lies between 0.09% and 0.11%, except soon after a meal. With normal individuals, the ingestion of 50 g. of glucose in 150 ml. of water causes an increase, even to 0.18%, but the concentration falls back to normal in about 90 minutes. With diabetic patients there is usually a greater increase, but of more importance in diagnosis is a characteristic delay, often of many hours, before the sugar decreases to the usual value.

Renal Glycosuria. A glucose tolerance test shows a low or normal blood sugar curve but is accompanied by glycosuria. There is a lowering of the renal threshold which is not of proved pathological significance and no treatment is required.

Glycolysis. Sugar tends to disappear slowly from drawn blood and unless the blood is to be examined immediately some form of preservative must be used. For this purpose a mixture of sodium fluoride 1 g. and thymol 0.1 g. is the most suitable. It is used in the proportion of 0.1 g. to 10 ml. of blood. It acts both as an anti-coagulant and preservative. Arterial blood has a slightly higher figure than venous blood and therefore blood collected by finger or ear prick will show a sugar content of a few mg. % above that in blood collected by vein puncture.

Maclean's Method. 0.2 ml. of the blood is mixed with 23.8 ml. of a solution containing 15% of sodium sulphate and 0.1% v/v of acetic acid. After raising to boiling and removing from the flame, 1 ml. of dialysed iron solution is added, and, when cooled, the mixture is filtered through a Whatman paper, 20 ml. of the filtrate being transferred to a 100 ml. conical flask. 2 ml. of standard copper solution (see below) is added and the mixture boiled for 6 minutes, cooled, and acidified with 2 ml. of 25% sulphuric acid. In one minute the liberated iodine is titrated with fresh N/400 sodium thiosulphate, using two drops of 1% soluble starch solution towards the end. The standard copper solution is also similarly titrated against N/400 thiosulphate by adding 2 ml. of 25% sulphuric acid to a mixture of 2 ml. of the copper solution and 5 ml. of the acid sodium sulphate solution.

The difference between the two titration readings gives the ml. of thiosulphate due to the sugar, and the percentage is read off from a table (v. *Modern Methods*

in *Diagnosis and Treatment of Glycosuria and Diabetes*.—Maclean), or the amount can be calculated thus:—

$$\% \text{ sugar} = (A \times 0.049) + 0.012$$

where A = (ml. of N/400 thiosulphate per 2 ml. of copper solution) — (ml. of N/400 thiosulphate in experiment).

Standard Copper Solution. (Should stand a few days before use.) Potassium bicarbonate 12 g. is dissolved by gentle heat (not above 37°) in about 60 ml. of distilled water and potassium carbonate (anhydrous) 8 g. is added. Copper sulphate (cryst.) 0.35 g. dissolved in a little water, is mixed with this, and when effervescence is over, after warming, if necessary, to dissolve any insoluble carbonate, potassium iodate 0.05 g. and potassium iodide 0.50 g. are added. The solution is then filtered through a starch-free paper and adjusted to 100 ml. When titrated as described, 2 ml. should require about 11 ml. of N/400 thiosulphate. For accurate work, the heating of this solution and the blood filtrate should be ensured by using the same burner and gas-pressure to bring the 22 ml. of solution in the flask to vigorous boiling in 100 seconds.

Calvert's Modification. The blood is collected in a small platinum capsule and weighed on a torsion balance. After removal of protein and treatment with alkaline copper solution, phosphomolybdic acid is added. Unreduced copper is decolorised and the cuprous oxide present dissolves, giving a deep blue solution, the colour being proportional to the sugar originally present. This solution is compared in a colorimeter with standard blue glass discs. It is claimed that the accuracy of the method is high.—*Biochem. J.*, 1923, 177. See also *Brit. med. J. Epit.*, i/1925, 10.

Kramer and Gittleman's Modification of Folin and Wu's method is simple—only 0.05 to 0.1 ml. of blood required. The blood is drawn into a fine pipette and mixed with 1.5 ml. of distilled water. Proteins are precipitated by adding 0.1 ml. of 10% sodium tungstate solution followed by 0.1 ml. of 2/3N H_2SO_4 . This is well mixed, allowed to stand and centrifuged. The supernatant liquor is pipetted off and transferred to a Folin-Wu tube. Two controls are prepared with standard sugar solution, alkaline copper solution is added to all three, the tubes are heated, and 2 ml. phosphomolybdic acid solution added to each. The contents are mixed and the colour compared. A calculation shows the sugar in mg. per 100 ml.—*Pharm. J.*, i/1924, 140.

Folin and Wu Method. Transfer 2 ml. of tungstic acid blood filtrate to a Folin-Wu sugar tube graduated at 25 ml. To other similar tubes add 2 ml. of standard sugar solutions containing 0.2 and 0.4 mg. respectively of glucose. To each tube add 2 ml. of alkaline copper solution. Transfer the tubes to a rapidly boiling water-bath and heat for 8 minutes. Cool in running water. Add to each tube 2 ml. of phosphomolybdic acid reagent. After one minute dilute to the mark with water and mix thoroughly. Compare in a colorimeter, using the standard most nearly matching the unknown.

Calculation:

$$\frac{S}{U} \times 0.02 \times \frac{100}{0.2} = \text{mg. of glucose per 100 ml. of blood.}$$

The unknown is set at 20 mm. and the result obtained by multiplying the standard reading by 5. For high blood sugars use the stronger standard, and with the unknown set at 10 mm. obtain the result by multiplying the standard reading by 20.

The reagents used are: (1) *Alkaline Copper Solution.* Dissolve 40 g. of pure anhydrous sodium carbonate in 400 ml. of water; add 7.5 g. of tartaric acid, dissolve, and add 4.5 g. of crystallised copper sulphate; mix and make up to a litre. (2) *Phosphomolybdic Acid Solution.* To 35 g. of molybdic acid and 5 g. sodium tungstate add 200 ml. of 10% sodium hydroxide and 200 ml. of water. Boil for 20 to 40 minutes to remove most of the ammonia present in the molybdic acid. Cool, dilute to 350 ml., add 125 ml. of 85% phosphoric acid, and dilute to 500 ml. (3) *Standard Sugar Solutions.* (i) Stock solution containing 1% glucose in saturated benzoic acid solution; (ii) solution containing 2 mg. of sugar in 1 ml.; (iii) solutions containing 0.2 and 0.4 mg. of sugar in 2 ml.

This is the original Folin-Wu method (*J. biol. Chem.*, 1919, 38, 81; 1920, 41, 367); it has been subjected to numerous modifications (*J. biol. Chem.*, 1926, 67, 357; 1929, 82, 83), but is still widely used. For a description of the Folin-Wu Improved Method see Stitt; for a description of the Micro-Method of Folin and Wu see Harrison

Hagedorn and Jensen Method. This is a micro-titration method in which the blood protein is precipitated with zinc hydroxide, the filtrate heated with potassium ferricyanide solution and the amount of ferricyanide reduced determined by adding an iodide solution and titrating the iodine set free with sodium thiosulphate.

Sulphate

Normal human serum contains on an average 1.04 mg. of inorganic sulphate (calculated as S) per 100 ml., the content of whole blood being about twice this figure. The inorganic sulphate figure is increased in nephritis with retention and in leukæmia, diabetes, and intestinal obstruction.

Letonoff and Reinhold's Method. The following method, depending upon the colour given with benzidine in alkaline solution and β -naphthoquinone-4-sulphonate is stated to give accurate results. 6 ml. of a 0.4% aqueous solution of uranium acetate is added to 2 ml. of non-hæmolyzed serum in a centrifuge tube, and after thorough mixing the whole is centrifuged for 10 minutes. 4 ml. of the clear supernatant liquid is mixed in a centrifuge tube with 1 ml. of glacial acetic acid and 9 ml. of a 1% w/v solution of benzidine in acetone, the whole cooled in ice for 30 minutes and then centrifuged for 15 minutes at 3000 r.p.m. The supernatant liquid is decanted, the tube drained, 14 ml. of acetone added to the precipitate and the whole again centrifuged for 15 minutes, the acetone decanted and the tube drained. 1 ml. of a 1% solution of sodium borate in N/10 sodium hydroxide is added and the precipitate dissolved, if necessary by warming; finally 10 ml. of water and 1 ml. of a 0.15% aqueous solution of sodium β -naphthoquinone-4-sulphonate are added, and the colour compared in a colorimeter with standards prepared at the same time from 2 and 5 ml. of a 0.0803% aqueous solution of benzidine hydrochloride, equivalent, respectively, to 0.02 and 0.05 mg. of sulphur. The inorganic sulphur of normal human serum, thus determined, averages 1.04 mg. per 100 ml. The method is applicable also to the determination of sulphate in urine. —*J. biol. Chem.*, 1936, 114, 147.

Urea

The normal blood urea figure is 20–40 mg. per 100 ml. of whole blood, plasma or serum. The concentration of urea in the blood is very little influenced by ordinary mixed meals, but it is sometimes extensively affected by a great reduction of protein in the diet. In a proved kidney case, a rise in the blood urea indicates extensive renal dysfunction; in uræmia the figure may gradually rise to 800 mg. per 100 ml. immediately before death. Non-renal causes of an increase in the urea content of the blood, e.g., 50–100 mg. per 100 ml., are severe anhydræmia (diarrhœa, gastro-enteritis) and a failing circulation with fall in blood pressure which results in a diminution of urinary secretion. In this type of ætiology the concentration of urea in the urine is normal or high.

Blood urea may be most conveniently estimated by the micro-method described by Archer and Robb (*Quart. J. Med.*, 1925, 274). As an aid in diagnosis, estimation of the concentration of urea in the urine should be performed at the same time. (*For a description of the Van Slyke Urea Clearance Test, see p. 664.*)

Urease-Nesslerisation Method (Archer and Robb). In a non-tapered centrifuge tube place 2 ml. of distilled water and 0.2 ml. of blood, which is measured with an accurate blood pipette calibrated "to contain," and is delivered beneath the 2 ml. of water. The pipette is then raised and washed out with the water two or three times. Add 0.2 ml. of the urease suspension (prepared by grinding up one urease tablet in 5 ml. of 30% alcohol) and shake

well to mix. Place the centrifuge-tube in a water-bath at 55° for fifteen minutes (or in a beaker of water which has been heated to 60°). Remove the tube and add 0.3 ml. of 10% sodium tungstate, 0.3 ml. of two-thirds normal sulphuric acid and 5 ml. of distilled water. Shake well to mix, stand a few minutes till the protein precipitate flocculates and centrifuge till the supernatant fluid is quite clear. Pipette off 5 ml. of the supernatant fluid into a clean test-tube, and add 5 ml. of water and 2 ml. of Nessler's reagent.

The standards are prepared by mixing (A) 1 ml. of ammonium sulphate standard solution, 9 ml. of water and 2 ml. of Nessler's reagent, and (B) 2 ml. of ammonium sulphate standard solution, 8 ml. of water and 2 ml. of Nessler's reagent.

A solution of ammonium sulphate is kept in stock which has been prepared by dissolving 2.2 g. of the pure dry salt in water and making up to 1000 ml. The ammonium sulphate standard solution is prepared by diluting this stock solution 1 in 20 by taking 5 ml. and making up to 100 ml. with water.

Compare the colours of the "unknown" and of the standards with the unaided eye, and select the standard approximating most nearly to the unknown. Place the unknown in the left-hand cup of the colorimeter and set the reading at 40 mm. Place the selected standard in the right-hand cup and move up and down in the usual way till the colours match. Take a series of readings, of which let the average be S.

Calculation. The reading of the unknown is 40, that of the standard is S. Standard A corresponds to 40 mg. of urea per 100 ml. Standard B corresponds to 80 mg. of urea per 100 ml. Therefore,

$$\text{Blood urea} = \frac{S}{40} \times 40 = S \text{ mg. per 100 ml. (A)}$$

$$\text{or,} \quad \frac{S}{40} \times 80 = 2S \text{ mg. per 100 ml. (B).}$$

Nessler's Reagent. (1) Double Iodide Solution. Dissolve 150 g. of potassium iodide in 100 ml. of distilled water. Add 200 g. of mercuric iodide, HgI_2 , and wait till solution is complete. Then dilute to 1000 ml. with distilled water and filter. Dilute the filtrate to 2000 ml. (2) 10% Sodium Hydroxide. Prepare a saturated solution of sodium hydroxide (about 55%) by adding an excess of NaOH to about 200 ml. of water, and stopper securely. After two or three days decant the clear supernatant fluid, and dilute with distilled water to 10%. (Add 45 ml. of water to each 10 ml. of supernatant fluid.) Check the concentration of the sodium hydroxide by further diluting 10 ml. to 25 ml. with distilled water, and titrating 10 ml. of the supposed 4% NaOH with N/1 acid. If the concentration differs from the theoretical by more than $\pm 5\%$ (i.e., if in the titration 10 ml. of the sodium hydroxide requires more than 10.5 ml. or less than 9.5 ml. of N/1 acid) it must be adjusted. **Preparation of Nessler Reagent:** 10% sodium hydroxide 700 ml., double iodide solution 150 ml., distilled water 150 ml.

Chemical Spot Test in Diagnosis of Uræmia. To Ehrlich's aldehyde reagent add 20% trichloroacetic acid with shaking until the cloudiness forming with each drop just faintly persists. To a small volume of blood in a narrow test-tube add an approximate volume of the reagent. Shake vigorously for a few seconds and then pour a drop of the coagulum on to a white filter paper. The filtrate spreads in an extending circle leaving a brown centre of precipitated protein. With true uræmia, the spot external to this dark centre is a distinct green colour; when dry, the yellow colour of this spot slowly intensifies, becoming a bright canary yellow after several hours, while bloods with only a little or no urea retention leave only a dull pale green spot. Not a substitute for blood-urea estimation but suitable for emergencies to ascertain whether or not there is gross retention of nitrogen.—J. Patterson, *Lancet*, i/1934, 1061.

Andrewes' Simplified Diazo Test for Uræmia is carried out by removing the proteins from the serum by adding 2 vols. of absolute alcohol and centrifuging or filtering. To 4 vols. of the filtrate is added 1 vol. of diazo reagent—this is the same as that used in Van den Bergh's Test, *q.v.*—and the mixture boiled for $\frac{1}{2}$ to 1 minute when 40% soda solution is added drop by drop, shaking after each addition. The test is positive only when a deep pink or cherry red colour is seen, which colour may last only for a few seconds.—G. A. Harrison and L. F. Hewitt, *Brit. med. J.*, ii/1927, 1138. (This reaction is due to a high concentration of indican in the blood.)

Uric Acid

Normal blood contains from 2 to 4 mg. of uric acid per 100 ml. This figure is raised in advanced chronic nephritis, but there is no close relationship between the height of the blood uric acid and the degree of renal insufficiency. In most cases of gout there is usually a moderate increase of from 4 to 10 mg. and high figures have been reported in the toxæmias of pregnancy, in leukæmia, pneumonia, hypertension, arteriosclerosis, and other conditions. The figure tends to be on the low side during pregnancy and delivery, and may increase slightly after delivery. Estimations of uric acid are most frequently made in gout or suspected gout and for the purpose of following the effects of treatment of gout by drugs which cause a lowering of the blood uric acid, though according to Stitt, uric acid estimations have relatively little practical value. (*For a description of Folin's and Benedict's methods of determination, see Hawk.*)

A new method depending upon the change in colour of blood brought about by the action of uricase is described. The errors of the method tested by recovery studies on pure uric acid solutions were found to range from -2.8 to +5.1%.—M. B. Blanch and F. C. Koch, *J. biol. Chem.*, 1939, 130, 443.

OTHER SUBSTANCES IN BLOOD

Ascorbic Acid. A content of 0.7 mg. or more of ascorbic acid per 100 ml. of serum may be considered satisfactory. If only 0.3 to 0.7 mg. per 100 ml. the intake is suboptimal, and if less than 0.3 mg. there is a serious deficiency.—Minot *et al.*, *J. Pediat.*, 1940, 16, 717.

6 to 7 ml. of blood is run into a tube containing 5 mg. of potassium cyanide and 10 mg. of potassium oxalate. The tube is centrifuged and to 2 ml. of plasma are added 2 ml. of water and 6 ml. of fresh 10% metaphosphoric acid, and the mixture is stirred for 30 seconds. After standing for 3 minutes the mixture is filtered or centrifuged and 2 ml. of clear liquid is titrated with M/1000 dichlorophenolindophenol standardised against ascorbic acid. Values of 0.65 to 2 mg. % were found in 150 normal samples.—M. Pijoan and F. Klemperer, *J. clin. Invest.*, 1937, 16, 443.

The following is a more accurate and rapid method of estimation than that described by Farmer and Abt and modified by Pijoan and Klemperer. For the standard colour the following two solutions are required: Solution A (0.5 ml.) is added to Solution B (0.5 ml.) and the mixture is diluted to 35 ml. with freshly boiled and cooled distilled water. *Solution A:* This is a solution of methyl red in alcohol and is prepared as follows: methyl red 0.002 g.; N/20 NaOH 0.76 ml.; alcohol (90%) 4.0 ml. Dissolve in warm water-bath and dilute with alcohol (20%) to 200.0 ml. *Solution B:* This is a buffer solution, the composition of which is M/5 potassium chloride 50.0 ml.; N/5 hydrochloric acid 2.62 ml.; distilled water freshly boiled and cooled to 200.0 ml. The standard should be prepared daily. Comparison is made in pyrex tubes of 10 cm. length, 1.5 cm. external diameter, and 1.3 cm. internal diameter. The tubes are set at an angle of 55°. The standard colour is delivered from a burette so that the volume of solution in the standard tube is equal to that in the unknown.—Andrew Wilson, *Lancet*, i/1938, 667.

A simple method for Determination in Whole Blood. Place 2 ml. of oxalated whole blood in a test-tube of 2 cm. internal diameter and add 2 drops of octyl alcohol. Bubble coal gas through blood for 10 minutes, add 4 ml. glass-distilled water, continue passage of gas for 5 minutes more and then add 2 ml. of 32% metaphosphoric acid and mix by gassing for 30 seconds. Add 2 ml. sodium acetate solution and mix by tilting the tube. Transfer to a centrifuge tube and centrifuge. Titrate 2 ml. portions of the supernatant fluid, which should be crystal clear, in the centrifuge tubes, using a standard white light and white background. The indicator is measured from a 2 ml. microburette. A blank consisting of a 2 ml. sample of 6 ml. water, 2 ml. metaphosphoric acid and 2 ml. sodium acetate solution is titrated to a faint pink colour at the same time as the unknown.

Calculation: If X = titration figure for unknown and Y = titration figure for blank

$$\frac{5(X - Y)}{2} = \text{mg. of ascorbic acid per 100 ml. of blood.}$$

The reagents used are: (1) Solution of 2 : 6-dichlorophenolindophenol (1 ml. = 0.01 mg. ascorbic acid); (2) 32% solution of metaphosphoric acid; (3) 2N sodium acetate; (4) Octyl alcohol.—J. Deeny *et al.*, *Biochem. J.*, 1942, 36, 271.

Bromide. 2 ml. of serum is mixed in a test-tube with 4 ml. of water and 1.2 ml. of 20% trichloroacetic acid. The mixture is well shaken, allowed to stand 10 minutes and filtered through a 7 cm. Whatman No. 1 filter paper; 2 ml. of filtrate and 0.4 ml. of 0.5% gold chloride solution are mixed in a special comparison tube and the colour compared with that of a series of standard tubes. The latter are prepared by dissolving 1.45 g. of dry sodium bromide in 1 litre of water. Quantities of this solution varying from 0.5 ml. to 9.5 ml. are diluted to 10 ml. with water and 5 ml. of each dilution is added to 1 ml. of 0.5% gold chloride solution and 1 ml. of 20% trichloroacetic acid. Each 0.5 ml. of bromide solution originally employed to make the standard represents 25 mg. % of sodium bromide, allowance being made for loss of bromide in the sample under test owing to absorption by the precipitated protein.—R. F. Barbour, F. Pilkington and W. Sargent, *Brit. med. J.*, ii/1936, 957.

Lead. The normal lead content of blood varies from 30 to nearly 90 μg . Pb per 100 ml. Raised concentrations indicate an increased absorption of lead, but by no means constitute definite evidence of plumbism. Their greatest value is in assessing the degree of exposure to lead in industry, so that working conditions may be improved.—J. N. M. Chalmers, *Lancet*, i/1940, 447.

For a description of Tompsett and Anderson's Method for determination of lead in blood see p. 668; see also E. S. Wilkins *et al.*, *Industr. Engng Chem. (anal. Edn.)*, 1935, 7, 33; C. E. Willoughby, *ibid.*, 285. A method for the detection of punctate basophilia in blood is given on p. 677.

Sex Hormones. Androgens occur in the blood in such minute quantities that quantitative assays at the moment seem impossible. Attempts to assay the oestrogen content of blood have been made but the limited quantity of blood which may be drawn for analysis and the small quantity of oestrogen to be assayed make this problem extremely difficult.—R. G. Gustavson and F. E. D'Amour (Council on Pharmacy and Chemistry, A.M.A.), *J. Amer. med. Ass.*, ii/1941, 188.

Sulphonamides. For therapeutic purposes a level of 8 to 15 mg. of the free sulphonamide per 100 ml. of blood is the usual concentration aimed at. The total sulphonamide (i.e., free plus conjugated) is usually 20 to 50% more than the free. (For the most recent laboratory procedures see "The Medical Use of Sulphonamides," *Med. Res. Council, Lond., War Memo. No. 10*, 1943.)

Marshall's Method. The following reagents are required:—(1) 20% *p*-toluene-sulphonic acid in water; (2) freshly prepared 0.1% sodium nitrite; (3) 0.4% dimethyl- α -naphthylamine in 95% alcohol (stored in a dark bottle); (4) 0.05% saponin in water; (5) N/1 hydrochloric acid; (6) 2N sodium hydroxide; (7) 0.1% phenolphthalein in alcohol; (8) stock standard sulphanilamide, 20 mg. % in water. Working standards of 1, 0.5 and 0.2 mg. % are made from the stock standard by dilution. For estimation in blood, the working standard should contain also 18 ml. % of the *p*-toluenesulphonic acid solution. N.B.—Use all-glass cups in the colorimeter.

FREE SULPHANILAMIDE. 14 ml. of saponin solution and 2 ml. of blood are mixed, and after 2 minutes 4 ml. of *p*-toluenesulphonic acid solution is added with shaking. The mixture is allowed to stand for 5 minutes and then filtered.

Into a small tube measure 10 ml. of filtrate (equivalent to 1 ml. of blood) and 1 ml. of nitrite solution, shake and allow to stand for 3 minutes.

Run in from a burette 5 ml. of the dimethyl- α -naphthylamine solution. The colour produced is compared with that given by treating similarly 10 ml. of working standard solution, comparison being made in between 10 and 60 minutes. The 1 mg. % standard is satisfactory for blood between 5 to 20 mg. %.

CONJUGATED SULPHANILAMIDE. 2 ml. of blood, 30 ml. of saponin solution and 8 ml. of *p*-toluenesulphonic acid solution are mixed and filtered as above. Free sulphanilamide is determined on 10 ml. of the filtrate as described above. Another 10 ml. of filtrate is transferred to a test-tube marked at 10 ml. and is heated in a boiling water-bath for 90 minutes. It is then cooled and diluted to

10 ml. with water and the determination completed as described above. This gives total sulphanilamide.—*J. biol. Chem.*, 1937, 122, 263.

King's Method. This method is adapted from the procedure of Marshall (1939). 0.2 ml. of blood is added to 3.2 ml. of water or isotonic sodium sulphate; 0.6 ml. of 25% trichloroacetic acid is added. The mixture is vigorously shaken, and filtered or centrifuged; 2 ml. of the filtrate (= 0.1 ml. of blood) is transferred to a test-tube and 1 drop of sodium nitrite solution added. The tube is shaken and left for 3 min.; 1 ml. of ammonium sulphamate solution is added and the mixture left for 2 min. with occasional shaking. 2 ml. of naphthyl ethylene diamine solution is now added and the mixture shaken. The coloured solution is compared with a standard prepared in the same way from 2 ml. of standard solution (= 0.004 mg. sulphanilamide). The use of a yellow-green light filter (e.g., Ilford spectral yellow-green) facilitates the comparison.

Total sulphanilamide is determined by heating 2 ml. of filtrate with 0.5 ml. of N hydrochloric acid in a 5 ml. volumetric flask in a boiling water-bath for 1 hour. The cooled contents of the flask are then treated with sodium nitrite, etc., as in the procedure for free sulphanilamide, and the volume adjusted to 5 ml. with water.

Calculation:

$$\text{Blood sulphanilamide (mg. per 100 ml. blood)} = \left\{ \begin{array}{l} \frac{\text{Reading of standard}}{\text{Reading of test}} \times 0.004 \times \frac{100}{0.1} \\ \frac{\text{Reading of standard}}{\text{Reading of test}} \times 4 \end{array} \right.$$

If the colour of the test is more than twice as strong as that of the standard, the determination should be repeated with 1 ml. of filtrate plus 1 ml. of water.

If sulphapyridine or sulphathiazole is determined by the above procedure, and with a sulphanilamide standard, the value obtained is multiplied by 1.4 to give the mg. sulphapyridine or sulphathiazole per 100 ml. blood.

The reagents used are: 25% *Trichloroacetic Acid*.—25 g. of the acid dissolved in water and made up to 100 ml. *Sodium Nitrite Solution*.—0.5 g. dissolved in 100 ml. of water. This solution is the same as solution B in the bilirubin method. *Ammonium Sulphamate Solution*.—0.5 g. dissolved in water and made up to 100 ml. *Naphthylethylenediamine Solution*.—0.5 g. of N(1-naphthyl) ethylene diamine dihydrochloride in 100 ml. of water; stored in a brown bottle. *Stock Standard Sulphanilamide* (0.1 mg. per ml. 100 mg. of sulphanilamide is dissolved in 1 litre of water). *Standard Solution* (0.002 mg. sulphanilamide per ml.). 2 ml. of the stock standard, together with 15 ml. of the 25% trichloroacetic acid, is diluted to 100 ml. with water.—*Lancet*, i/1942, 207.

Fuller's Method. The following method takes only a few seconds, needs only a drop of blood and no special apparatus, is economical with reagents and gives results accurate enough for clinical use.

10 c.mm. of blood is placed on a paraffin block or waxed slide, 10 c.mm. of precipitating fluid added and mixed with a fine stirrer. A piece of test-paper about $\frac{1}{2}$ inch square is slid along and applied with a needle to the edge of the drop. The clear exudate is soaked up by the paper and the yellow colour produced compared with a series of standard papers. The comparison must be made quickly while the paper is still wet. Up to a strength of 10 mg. per 100 ml. results correct to 1 mg. can be obtained. Above this strength the colour differences are less well marked and dilution is advised.

Precipitating Fluid. This consists of 1 part of aqueous 50% *p*-toluenesulphonic acid, 3 parts of aqueous 20% phosphoric acid and 4 parts of alcohol.

Test papers are prepared by pouring a mixture of equal parts of a freshly-made 5% alcoholic solution of *p*-dimethylaminobenzaldehyde and pH 1.4 buffer on to Whatman No. 1 filter paper, which is dried, cut into strips and stored in a closed bottle. The pH 1.4 buffer is prepared from 10 ml. of N HCl and 10 ml. of 7% NaCl diluted to 250 ml.

Standard papers are prepared by dipping filter papers in concentrations of tartrazine. It is convenient to make up a 0.52% solution of tartrazine (= 50 mg. per 100 ml. sulphanilamide) and to dilute 1, 2, 3 ml., etc., to 50 ml. to get the various standards.—A. T. Fuller, *Lancet*, i/1942, 760.

Tocopherol. To 10 ml. of the serum in a 250 ml. separating funnel add, with gentle shaking after each addition, 5 ml. of 0.2N potassium hydroxide solution, 15 ml. of 37% formaldehyde solution (neutralised to phenolphthalein) and 15 ml. of alcohol. 50 ml. of peroxide-free ether are added, the mixture is thoroughly

shaken and allowed to stand. The aqueous layer is extracted twice more with 50 ml. of ether after the addition of 10 ml. of alcohol each time. The combined ethereal extracts are washed first with 25 ml. of 2% potassium hydroxide solution, then with 25 ml. of 1% sulphuric acid, and finally several times with water. The extract is dried over anhydrous sodium sulphate, mixed with 10 ml. of benzene, and evaporated under reduced pressure in an atmosphere of carbon dioxide. A further 10 ml. of benzene are added to the residue in the flask, and the mixture again evaporated. The small residue is taken up in 5 ml. of benzene and filtered through a column of "Floridin XS" earth. The column is washed with 25 ml. of benzene and the filtrate evaporated under reduced pressure. The residue in the flask is dissolved in 5 ml. of the reagent mixture (1 ml. of dipyriddy solution, 1 ml. of ferric chloride solution and 5 ml. of benzene made up to 25 ml. with alcohol), and the colour of the mixture measured after 10 minutes in a Pulfrich photometer. Acetyl-tocopherol, also extracted by this method does not react with the reagent unless it is hydrolysed. This may be effected by heating at 72° to 74° with methyl alcoholic potassium hydroxide solution for ten minutes, diluting with water and extracting with three 50 ml. portions of ether. In estimating acetyl-tocopherol, a compromise has to be sought between the quantity of alkali necessary for saponification and the concentration necessary to reduce destruction of the tocopherol to a minimum. —A. Emmerie and C. Engel, per *Analyst*, 1939, 837.

CEREBROSPINAL FLUID

The composition is virtually that of Locke's modification of Ringer's Solution. In examining a specimen, centrifuge or allow to stand for any sediment to deposit. Examine sediment for cells and bacteria. Inoculate broth and other media for bacteria.

Normal cerebrospinal fluid is a clear colourless fluid with no clot and should have the following characteristics:

Reaction: faintly alkaline.

Cells: Not more than 5 per cu. mm.

Total Protein: 10 to 35 mg. per 100 ml.

Globulin test: Negative.

Sugar: 40 to 80 mg. per 100 ml. (The sugar content will vary with the level of the blood sugar).

Chlorides (as NaCl): 700 to 750 mg. per 100 ml.

Urea: 10 to 40 mg. per 100 ml. This will vary with the blood urea.

If the fluid contains a clot this indicates the presence of fibrinogen and is always of pathological significance unless blood has been admixed at the time of the lumbar puncture. If the fluid is red the colour is due to blood. Red blood cells may be detected in the centrifuged deposit of an apparently colourless fluid. A yellow fluid is due to bilirubin formed from the hæmoglobin of a previous hæmorrhage.

Estimation of Total Protein. This is best carried out by Mestrezat's method. The protein is precipitated by 30% trichloroacetic acid and the resulting turbidity is compared with a series of standard protein solutions similarly treated. (See Harrison for full description.) Nearly all pathological fluids show an increase of protein. This increase of protein may or may not be accompanied by an increase of cells. Bacterial infection usually results in an enormous increase of cells. When there is a block in the subarachnoid space the protein content may be very high. A high protein content without an equivalent cell increase is known as *Froin's syndrome*. This occurs in tumours of the cord, spinal caries and chronic meningitis.

In interpreting the results of protein estimations it is important to realise that the normal amount of total coagulable protein varies, not only with the age of the patient but with the level at which the specimen is drawn off from the cerebrospinal system. It is least in amount in specimens from children and from ventricles, and greatest in elderly patients and in lumbar puncture fluids, whilst cisternal fluids give intermediate readings.—W. E. C. Dickson, *Med. Pr.*, 1937, 79.

Qualitative Test for Globulin. The test usually employed is that of adding a small quantity of cerebrospinal fluid to an equal quantity of saturated ammonium sulphate. After shaking, normal fluids remain clear or show only the faintest degree of opalescence. An increased globulin content is always pathological if there is no blood present in the specimen. Protein figures and globulin tests in a specimen containing blood are of no value. The chief value of the globulin test is in syphilitic lesions where although the total protein may be only slightly increased the globulin ratio is so raised that definite positive reactions are obtained.

Sugar. This varies with the blood sugar but tends to lag a little behind. In acute meningitis the sugar is often lowered.

Chlorides. These may be estimated by direct titration with N/50 AgNO_3 , using potassium chromate as the indicator. High figures are not often met with but occur in advanced cases of renal inefficiency. Low chloride figures are found in meningitis. Chloride estimations are particularly valuable in the early diagnosis of tuberculous meningitis, since the figures in this disease are usually very low, between 500 and 650 mg. Any factor tending to lower the blood chloride may cause a fall in the chloride content of the cerebrospinal fluid.

DETERMINATION—USING DICHLOROFLUORESCEIN INDICATOR. 1 ml. of the filtered fluid is added to 15 ml. of water and 5 ml. of sodium acetate-acetic acid buffer solution to bring the pH to 5, followed by 2 ml. of dichlorofluorescein solution and the whole titrated with silver nitrate solution until the precipitate is pink. This method is also applicable to gastric juice and milk.—C. F. M. Rose, *Biochem. J.*, 1936, 1140.

INTERPRETATION OF RESULTS. An increase above 750 mg. usually indicates cardiac and renal insufficiency and deficient elimination of chlorides by the kidneys; whilst a fall below 720 mg. is usually due to acute meningitis, in which it may drop to 680, 650 or 630 mg. An even more marked fall to 580 mg. or less may occur in tuberculous meningitis; whilst on the other hand in "aseptic lymphocytic meningitis" and other virus diseases, including poliomyelitis, the chlorides and sugar are not reduced in amount.—W. E. C. Dickson, *Med. Pr.*, 1937, 81.

A sodium chloride content of 550 mg. per 100 ml. may be considered practically pathognomonic of tuberculous meningitis. Microscopical examination of such a fluid, if sufficiently prolonged, will always reveal tubercle bacilli. Only about 2% of cases of tuberculous meningitis may show a high chloride content (over 600 mg. per 100 ml.), and these will fail to reveal organisms even after 2½ hours' search. (The technique of the test—a modification of that described by Whitehorn—is given in detail; its performance occupies 15 minutes.)—J. Ingham, *Brit. med. J.*, ii/1937, 111.

Urea. This always bears a close relationship to the level in the blood, though tending to be a little lower. There is no special value in estimating the spinal fluid urea in cases of suspected uræmia.

Sugar and urea may be estimated by the methods employed in the analysis of blood.

Differential Diagnosis of Syphilitic from Parasyphilitic Affections by Examination of Cerebrospinal Fluid.

Normally, the fluid is practically free from corpuscular elements; from 1 to 5 lymphocytes may be seen in the centrifuged deposit in the ordinary microscopic field. In acute microbial infections of the cerebrospinal meninges leucocytosis occurs, mostly of the polymuclear type.

In certain more chronic affections, as in tuberculosis, trypanosomiasis and syphilis, excess of leucocytes also occurs—mostly *mononuclear*, i.e., there is a lymphocytosis or pleocytosis. Tubercle bacilli and trypanosomes can usually be found, but the *Sp. pallida* has not been found. The pleocytosis of cerebrospinal syphilis, tabes and general paralysis is often a very early occurrence of great diagnostic value.

In florid syphilis and in cerebrospinal syphilis as well as in tabes and general paralysis, the Wassermann reaction is practically always +.

The second reaction in *diagnosis of parasyphilitic affections* is the finding of an excess of globulin. In combination with a + Wassermann reaction and pleocytosis it is pathognomic of parasyphilis.

The third reaction is the pleocytosis; the fourth is the Wassermann reaction, both already mentioned. The four reactions are relied upon for diagnosis.

Routine examination of cerebrospinal fluid in syphilis.—C. H. Mills, *Brit. med. J.*, ii/1927, 527. See also J. G. Greenfield, *Lancet*, ii/1928, 716.

Colloidal Gold Reaction. Zeigmondy found that certain colloids exerted definite degrees of protective action on the precipitation of gold suspensions by sodium chloride. Lange applied the test to the fluid protein and found that normal fluid when diluted with a 0.4% sodium chloride solution does not affect the solution of colloidal gold, whilst in disease of the central nervous system characteristic changes are produced.

All cerebrospinal fluids precipitate colloidal gold provided that the gold solution is sufficiently sensitive, but, generally speaking, the precipitating substance is contained to a small degree in normal fluid, to a greater degree in tabetic cases, and to a still greater degree in paretic cases.—*Rep. med. Res. Com.*, 1919-20.

Technique. Colloidal gold solution is red. Numbers are given to the colours formed on mixing the specimens with the gold; red=0, red-blue=1, violet=2, blue=3, bluish-white=4, and colourless=5. Ten dilutions are made, varying from 1/10 to 1/5120. Results are given from left to right. In general paralysis a typical reading would be 5555554210 (the "paretic curve"). In tabes the figures may be 2221110000. In cerebrospinal syphilis 1223320000 is fairly typical.—A. Douglas Bigland, *Lancet*, ii/1920, 587.

The criteria of the suitability of colloidal gold for testing cerebrospinal fluid are: (1) It must give a paretic curve with fluid from a case of general paralysis. (2) Show no change with normal fluid. (3) 5 ml. should be completely precipitated in 1 hour by 1.7 ml. of a 1% sodium chloride solution. (4) It should be neutral to 1% alizarin red in 5% alcohol.—D. K. Adams, *Lancet*, i/1921, 420.

Cases of disseminated sclerosis give a positive curve in about 30% of the number of fluids tested.

In 20 cases of functional nervous disease the cerebrospinal fluid gave negative results. Apparently not specific, but of value to indicate first definite sign of involvement of the central nervous system in organic disease.—D. K. Adams, *Brit. med. J.*, ii/1921, 842.

The reaction is of value for differentiating one pathological condition from another, rather than as was first expected for making a quantitative determination of the protein.—*Physiology and Pathology of Cerebrospinal Fluid*, W. Boyd, 1920.

Preparation of the Gold Solution for the Reaction.

To 100 ml. of triple-distilled water add 1 ml. of 1% solution of gold and sodium chloride ($\text{AuCl}_3 \cdot 3\text{NaCl}$). Bring to the boil and add 10 drops of 1% formalin. Remove partially from the flame and add 16 drops of 2% potassium carbonate solution and then at intervals of 15 seconds one or two more drops; 18 usually suffice. The colour should be a "smart bluish," changing to old rose as it cools, with fluorescence. 5 ml. will be rapidly decolorised by 1.7 ml. of 1% sodium chloride, and it will give luetic and paretic curves with appropriate positives, and negative results with known negatives. Should not be used quite fresh but on the other hand it may not keep more than a week.—T. Grey, *Brit. med. J.*, ii/1922, 1120; i/1923, 88.

Gettler and Jackson's method.—*Yearb. Pharm.*, 1922, 38.

A simplified method: One ml. of 1% gold chloride and 1 ml. of 2% potassium carbonate are added to 100 ml. of water. This is heated and as it begins to boil 1 ml. of 0.5% glucose is added, and the boiling continued. Fluid turns violet in a minute, and then purple, when it is removed for use.—*Yearb. Pharm.*, 1922, 38.

Benzoin Reaction for Syphilitic Disease of the Central Nervous System.

One gramme of freshly powdered sumatra resin dissolved in 10 ml. of absolute alcohol; shake well and leave for 48 hours. Decant and add 0.3 ml. of the clear fluid to 20 ml. of twice distilled water at 35°. For each fluid to be examined, a series of 6 or 12 test-tubes is put up. Into the first tube 0.25 ml. of sodium chloride solution (0.01%) is placed, and into the remaining

tubes 1 ml. of saline. 0.75 ml. of cerebrospinal fluid is measured into the first tube, and 1 ml. into the second tube; 1 ml. from the second tube is put into the third tube and mixed; 1 ml. from tube 3 is put into tube 4, etc., until the penultimate tube is reached, when, after mixing, 1 ml. is discarded; the last tube, containing only saline, acts as control. To each tube, including control, add 1 ml. of the gum benzoin suspension and mix; leave for 12 hours. In reading, three degrees of precipitation are recognised, 0, 1, and 2. In the first no change occurs; the second shows some precipitation, but fluid remains opaque; the third shows complete precipitation with clear supernatant fluid. "Negative" or "normal" readings, either no change whatever in all tubes, or precipitation in tubes 7 to 9 inclusive. A "positive" reading is complete precipitation in all tubes, with sometimes a slight deviation in tubes 11, 10, or 9, and sometimes the first tube remains opaque. Test nearly as sensitive as Wassermann to syphilis of the central nervous system. Positive result is not obtained with any disease other than syphilis of central nervous system.—J. A. Braxton Hicks and J. Pearce, *Brit. med. J.*, i/1924, 268.

Mastic Test (introduced by Emanuel) is on lines practically identical with the preceding except that mastic is used instead of benzoin.

In positive cases complete precipitation of the mastic occurs in a given number of tubes and results are read in the same way as in the colloidal gold test. When precipitation is complete the fluid becomes perfectly clear, and there is a heavy white deposit at the bottom of the tube.—*Physiology and Pathology of the Cerebrospinal Fluid*, William Boyd, 1920.

The benzoin and mastic tests are of approximately equal value, but the benzoin is simpler. In special cases, such as meningitis and multiple sclerosis, the colloidal gold test is preferable.—*J. Amer. med. Ass.*, ii/1925, 1584.

A Colour Reaction in General Paresis.

To 1 ml. of cerebrospinal fluid add 0.3 ml. of acetic anhydride. Shake well and add drop by drop 0.8 ml. of conc. sulphuric acid. Lilac tint indicates positive reaction; a brown-yellow or red-yellow tint a negative. Lilac tint appears immediately after addition of sulphuric acid, usually remaining about 5 minutes. Positive in 97% of cases of general paresis, and negative with almost every other type of mental disorder, except certain cases of neurosyphilis (other than general paralysis of the insane).—J. S. Harris, *Brit. med. J.*, i/1926, 136.

Tryptophane Test. This test is of value in the early diagnosis of tuberculous meningitis. It is suggested that the tryptophane is produced by tubercle bacilli in the cerebrospinal fluid. The test is carried out as follows: Mix 2 to 3 ml. of the fluid with 15 ml. to 18 ml. of B.P. hydrochloric acid and 1 to 2 drops of 2% solution of formaldehyde. In 5 minutes add slowly down the side of the tube 25 to 30 drops of a 0.06% solution of sodium nitrite. A positive reaction is shown by a violet ring being formed at the point of contact.

The test is inapplicable in purulent, hæmorrhagic, or xanthochromic fluids, as in these cases a false positive reaction is given; this appears as a purplish ring at the fluid junction, deeper in colour, thicker and more easily seen than the true reaction and in many cases diffusing into the fluids both above and below.—J. Spillane, *Lancet*, i/1937, 560.

FÆCES

Examination of fæces is undertaken to determine the state of the various digestive functions and is thus of assistance in the treatment of gastric and intestinal disease.

A trial diet is necessary. Ordinary meals are taken during 48 hours as follows: to include (1) milk undiluted or mixed with coffee; (2) eggs; (3) animal food, such as fish, poultry, veal, beef, etc.; (4) farinaceous foods—bread, potatoes, rice; (5) the various green vegetables and roots; (6) stewed fruit; (7) butter and various fats of meat.

The fæces are collected in a glass vessel—this permits macroscopic examination (in constipation, etc.).

Colour. The colour of normal stools varies considerably with the diet. A diet consisting chiefly of milk and carbohydrates will give pale yellow stools. A diet rich in meat will give dark brown stools. Black stools may be due to ingested drugs such as iron or bismuth, or to excessive bleeding, resulting in the tarry stool due to altered blood (melæna). Green stools of infants are usually due to bilirubin which owing to the rapid passage of the diarrhœic stool has escaped conversion into stercobilin. Stools of very young infants contain bilirubin and these on exposure to air may turn green by oxidation of the bilirubin to biliverdin. The normal pigment of a stool is stercobilin and in adults bilirubin is abnormal.

Test for Bilirubin. To a smear of fæces on a white tile add two drops of fuming nitric acid. A blue or green colour denotes the presence of bilirubin.

A more sensitive test is to add 2 drops of Fouchet's reagent to a smear of fæces on a white tile. A green or greenish-blue colour indicates the presence of bilirubin.

Stercobilin (Urobilin). This is present in all normal stools, but is absent or deficient where there is any obstruction to the passage of bile into the intestine.

Test for Stercobilin. Make a thick emulsion of the stool with amyl alcohol and allow it to stand for 12 hours. Decant the supernatant fluid and after adding two drops of tincture of iodine mix with an equal volume of saturated zinc acetate in alcohol, and filter. The filtrate will be yellow or pinkish-yellow by transmitted light but will show a marked green fluorescence by reflected light if stercobilin is present.

Fats. The fat content of normal fæces on an average diet is:—

Neutral fat: 6 to 7%.

Free fatty acids: 7 to 8%.

Fat as soaps: 8 to 10%.

All calculated on the dried stool.

Neutral fat is increased in pancreatic deficiency. Free fatty acids and acid soaps (split fat) are increased in biliary deficiency, intestinal hurry and abnormal conditions of the intestinal mucous membrane preventing absorption.

Estimation of Fat. Fat may be estimated by extracting 1 g. of dried stool in a Soxhlet's apparatus with ether. The ether extract is evaporated, dried and weighed. This weight is neutral fat and free fatty acid. The dried fats are re-dissolved in a little ether and after dilution with alcohol the free fatty acid is titrated with N/10 alcoholic KOH, phenolphthalein being used as indicator and each ml. of KOH being taken as equivalent to 0.0268 g. of fatty acid. The residue left after the first ether extraction is transferred to an evaporating basin and 10 ml. of 10% HCl in alcohol added (concentrated HCl 10, alcohol 100). This is placed on a water-bath and when reduced to about 5 ml. mixed with sufficient plaster of paris to make a paste. This is then carefully dried on the water-bath and the resulting powder replaced in the Soxhlet thimble and extracted with ether. The ether extract evaporated and weighed gives the amount of fat present in the form of soaps. Care must be taken not to confuse liquid paraffin and its numerous preparations with undigested fat.

An improved method for the determination of fat in fæces.—E. C. Wood and T. W. Simpson, *Analyst*, 1934, 817.

Protein. The only microscopic evidence of undigested protein in the stool is the presence of undigested muscle fibres. Many such fibres suggest pancreatic deficiency or possibly rapid passage through the small intestine.

Carbohydrate. Occasionally starch escapes digestion and appears in the stool. This may be recognised microscopically after the addition of a little iodine.

Fermentation. Set aside a portion in a fermenting flask. Distinct gas evolution in 12 hours shows that starch digestion has not been satisfactory. The fæces in this case are distinctly acid—catarrhal affections of the small intestine. Gas evolution after 24 hours or later shows that the albuminous substances are being split up by the increased alkalinity of the fæces. In the former case there is *intestinal fermentation dyspepsia*, and in the latter *intestinal decomposition dyspepsia*.

Blood. Occult blood in stools may be recognised by:

Benzidine Test. The patient should have been on a meat-free and green-vegetable-free diet for at least 3 days. Superficial bleeding from the anus must be excluded. Bleeding from gums or nose should be looked for.

A small fragment of stool is mixed with about 5 ml. of water in a test-tube and boiled on a water-bath for 5 minutes. After cooling, it is added to about 5 ml. of saturated solution of benzidine in glacial acetic acid to which 10 drops of 10 vol. hydrogen peroxide have been added. On shaking, a blue colour shows the presence of blood. The test is extremely sensitive, and faint or doubtful reactions should be ignored.

Ascorbic acid interferes with the benzidine test. It may be removed as described for urine (p. 670).

Meyer's Phenolphthalein Reagent. Phenolphthalein, 2 g., potassium hydroxide 20 g., water 100 ml. Dissolve and add zinc 10 g., and boil. Filter while hot (and decolorised). Keep in the dark with a little zinc at the bottom.

In using, a small piece of faeces is taken from the middle of the stool after a milk diet and made into a fine suspension by adding water. Fill a test-tube about one-third full with this. Add one-third of its volume of glacial acetic acid, mix, boil and cool under tap. Add 5 ml. of ether, mix well and set aside. Pipette off and add 1 ml. of the reagent and a few drops of hydrogen peroxide. If blood is present an *immediate* deep red colour spreads down the tube.

Blood in water gives a positive reaction 1 in 500,000. A slightly modified form of the test employed. Copper, even in traces, interferes with the test.—*J. chem. Soc. Abstr.*, ii/1922, 724.

Amidopyrine Test. Take 5 ml. of a boiled and cooled stool emulsion in water. Add 3 to 4 drops of acetic acid and then 2 ml. of 5% amidopyrine in alcohol so that a layer of the latter is present on the surface. Drop in 5 to 6 drops of 10 vol. hydrogen peroxide. A mauve colour spreading up into the amidopyrine layer will show the presence of blood. This test is a good one although not nearly so sensitive as the benzidine test.

Thymolphthalein Test. Dissolve thymolphthalein 1 in water 100 and add potassium hydroxide 25 and zinc powder 10. Boil until colourless, filter hot and make up to original volume. Keep zinc filings in the solution to prevent oxidation.

To use the test, rub down a small portion of the faeces (e.g. the size of a bean) with 5 to 10 ml. of alcohol and 20 drops of glacial acetic acid. 25 to 30 drops of the extract are filtered off and 20 drops of the reagent mixed with 15 drops of hydrogen peroxide added. On shaking, a greyish-blue opaque ppt. forms, turning blue on standing if blood is present.—*Yearb. Pharm.*, 1919, 46.

Spectroscopic Detection of Haematin or Porphyrin in Faeces. (Snapper's Method). Extract about 10 g. of faeces with acetone until the filtrate becomes practically colourless. To the faecal residue add 25 ml. of a mixture of 1 part glacial acetic acid and 2 parts ethyl acetate. Filter. Examine the filtrate spectroscopically for alkaline haematin and alkaline porphyrin. Divide into two portions. To the first add a quarter of its volume of pyridine and a few drops of fresh yellow ammonium sulphide. Examine at once, without shaking, for the bands of haemochromogen. To the second portion add half its volume of 10% hydrochloric acid and an equal volume of ether. Examine the aqueous layer for bands of acid porphyrin.

Microscopic Examination. The presence of *connective tissue and elastic fibres* indicates a defect in acidity of the gastric juice. Defective dissociation of connective tissue and coagulable proteins points to a primary gastric affection known as *achylia gastrica* (*Hayem's hypopepsia*). Appearance of elastic fibres, if not associated with connective tissue and coagulated protein, must be regarded as a sign of good gastric, but defective intestinal digestion. Considerable amount of undigested muscle fibre with well-marked contour may indicate bad intestinal digestion of meat.

Mucus. Stain smear with 1% sodium alizarine sulphonate. Normal mucus appears as small, faintly yellow flakes and scales. It is possible to determine the section of the intestine from which mucus is derived by the tint of this colour—the further the distance to the anus the mucus has to travel the lighter the colour.

Detection of trypsin in the faeces to assist diagnosis of pancreatic disease. Rub up a small quantity with glycerin, place on a serum plate and incubate at 55° to 60° for 24 hours; note occurrence of depression in the medium. The

reaction is not due to pepsin. The amount of ferment was found to be distinctly greater in loose stools or diarrhoea, indicating that probably owing to the increased peristalsis the reabsorption or destruction of ferment is hindered and an increased quantity voided.

PLEURAL AND PERITONEAL FLUIDS

Physical Characters.

Note whether blood-stained or not. (Caution: A small amount of blood may get into the fluid in the process of exploring).

Observe whether transparent or otherwise.

Test for fat.

Note the consistence, specific gravity, odour, amount and nature of deposit.

Chemical Investigation will give:—

(1) Reaction, (2) Presence of serum albumin and serum globulin, (3) Presence of mucin or nucleo-albumin by addition of acetic acid.

Microscopic Examination of Sediment. For blood, epithelial cells, cancer cells, Foulis' cells (these are met with in fluids from malignant ovarian cysts or malignant peritonitis following such cysts), hooklets, crystals, actinomycosis nodules, *Amœba dysenteriae*.

General Characters. It is difficult to tell a dropsical from an inflammatory fluid. It appears that the amount of proteins in an effusion depends much more upon site than upon cause. Pleural fluids contain the highest percentage of proteins, peritoneal fluids rather less and subcutaneous fluids very little. The fluid in cardiac dropsy is more highly albuminous than in dropsy of renal origin. Diagnostically all one can say is that a fluid with sp. gr. more than 1.018 containing more than 4% of albumin is almost certainly inflammatory while one with sp. gr. less than 1.015 and an albumin percentage less than 2½% is certainly dropsical. For further details see R. Hutchison and H. Rainy, *Clinical Methods*.

STOMACH CONTENTS

In a healthy subject food commences to pass the pylorus in from fifteen minutes to half an hour after ingestion, the time varying with the character of the food (e.g., carbohydrates leave the stomach before proteins), and the stomach is empty in five hours. The passage through the small intestine takes about three and a half to five hours, about one inch a minute, so that there is food in the cæcum before the whole meal has left the stomach.

Test Meals.

Ewald Test Meal. This consists of 2 oz. of toast without butter and half a pint of tea without milk. The meal is removed one hour after its ingestion by means of an œsophageal tube either by a syringe or a Senoran's bottle. This type of test meal has been replaced by the Fractional Test Meal.

Fractional Test Meal. The patient swallows a Ryle's tube and then drinks a meal of weak oatmeal gruel. This is made by adding 2 tablespoonfuls of oatmeal to a quart of water, boiling gently until the volume is reduced to a pint and then straining through muslin. Before taking the gruel the stomach is emptied by gentle aspiration with a syringe and the fluid examined as a

sample of resting juice. If there is any question of delay it is an advantage to give the patient a charcoal biscuit the night before the test. Any charcoal in the morning resting juice will be definite evidence of delayed emptying of the stomach. Samples of the gastric contents are withdrawn at half hourly intervals and each analysed for its HCl content, total acidity and total chloride content. A note is made of the appearance of any blood or bile in any specimen. The rate of emptying is judged by the disappearance of starch from any sample. It is usual to continue the examination up to two hours.

Examination of Sample of Gastric Fluid. The presence of blood, charcoal and bile can be detected by the naked eye. Chemical tests for blood are unsatisfactory as it is common to get positive reactions, probably due to traces of blood produced by trauma. Before beginning the chemical examination the specimen should be filtered.

Free Hydrochloric Acid. This can be determined by Gunzburg's test. Mix six drops of 10% phloroglucin in alcohol with half the quantity of 10% vanillin in alcohol. Place this in an evaporating basin and add two or three drops of the filtered specimen. Evaporate gently on a water-bath. If free hydrochloric acid is present the residue will assume a deep cherry colour.

Resorcinol may be used instead of phloroglucin. The colour produced is slightly more purple.

Response to dimethylaminoozobenzene may be given by organic acids. In gastric ulcer and hyperchlorhydria free hydrochloric acid is always present; in carcinoma it is scarcely ever present.

Boas' Test for Free Hydrochloric Acid. Resorcin 5, cane sugar 3, alcohol 100. This test is used exactly as Gunzburg's test, the same red colour being produced, but Boas' solution requires heating more carefully, as it chars more readily and the colour is not permanent.

Thymol Blue. Add a few drops of 0.1% thymol blue to 10 ml. of the test meal filtrate. A red colour shows the presence of free hydrochloric acid. The amount present can be determined by titrating this solution with N/10 NaOH until the solution becomes yellow at pH 2. Each ml. of N/10 NaOH is equivalent to 0.00365 g. of HCl.

Total Acidity. This can be estimated in the same solution by continuing the titration until the indicator becomes blue. The reading of the titration from the beginning until the blue end-point, pH 8.8, is reached is a measure of the total acidity. The result can be expressed in terms of hydrochloric acid or as the number of ml. of N/10 NaOH equivalent to 100 ml. of test meal.

Estimation of Total Chlorides. To the same 10 ml. of the filtrate add 15 ml. of N/10 AgNO_3 and 3 ml. of conc. HNO_3 . Heat on a boiling water-bath and add saturated solution of potassium permanganate until the brown colour goes with difficulty. This will indicate the almost complete destruction of organic matter. Cool and add a few drops of 10% ferric alum as indicator. Titrate the excess of silver nitrate with N/10 KCNS. The chloride may be expressed in terms of HCl or again as number of ml. of N/10 NaOH equivalent to 100 ml. of test meal.

Alcohol Meal. This is of value in cases of suspected pernicious anaemia where the important point is the presence or absence of free hydrochloric acid. 50 ml. of 7% alcohol are given and samples of the contents of the stomach aspirated in the usual way up to an hour. If at the end of this time no free HCl has appeared a hypodermic injection of 0.1 mg. of histamine is given and the half-hourly collection of specimens continued. If at the end of 2 hours there is no free acid it can definitely be taken as a condition of achlorhydria.

Definition of terms. The following classifications are suggested by Harrison:

| | Ewald Test Meal | Fractional Test Meal |
|----------------------------------|-----------------------|---|
| FREE HCl | | |
| Achlorhydria | Absent | Absent throughout |
| Hypochlorhydria | 1 to 19 ml. of N/10% | Never above 10 ml. of N/10% |
| Isochlorhydria (normal free HCl) | 20 to 60 ml. of N/10% | Ranges between 11 and 60 ml. of N/10% |
| Hyperchlorhydria | Over 60 ml. of N/10% | One or more points above 60 ml. of N/10% |
| ACIDITY | | |
| Anacidity | Absent | Absent throughout |
| Hypoacidity | 1 to 29 ml. of N/10% | Never above 20 ml. N/10% |
| Isoacidity (normal acidity) | 30 to 70 ml. of N/10% | 21 to 70 ml. of N/10% |
| Hyperacidity | Over 70 ml. of N/10% | Over 70 ml. of N/10% at one or more points. |

Achylia. Absence of free HCl and of pepsin with low chlorides, i.e., absence of gastric secretion.

Gastric function in health and disease.—J. A. Ryle, *Lancet*, i/1925, 583, 641, 697, 754.

The technique and clinical interpretation of the fractional method of gastric analysis.—L. M. Morris, *J. Roy. Nav. Med. Service*, 1928, 89.

The following are abstracts from the works of Willcox, Herschell, Martin and others:—

Chemical Examination of the gastric contents after a test meal containing little protein and nitrogenous bases.—Willcox.

The hydrochloric acid in this case will be present as far as possible in the free condition (which is the point of importance in diagnosis of gastric ulcer).

I. Total Acidity (Normally=0.15% HCl). Determine whether there is active hydrochloric acid or a mixture of this and organic acid. Usually in chronic gastritis acidity is low. In gastric ulcer it is high. In carcinoma it is usually low. (A normal acidity does not exclude gastric carcinoma.) It is increased in simple hyperchlorhydria, peptic ulcer, cholelithiasis, appendicitis, and colitis.

Without doubt both total acidity and free hydrochloric acid are raised in a considerable proportion of ulcer cases. Duodenal cases show on an average a larger and more constant increase of acidity than the ulcers on the gastric side.—*Brit. med. J.*, ii/1912, 940, *et seq.*

Congo Red Paper. The colour caused by organic acids will disappear on warming over a spirit lamp whilst that due to hydrochloric acid remains.

II. Hydrochloric Acid. This, according to Willcox, is either (a) *free*, (b) *combined* with protein and organic bases (i.e., *physiologically active*), or (c) *inorganically* combined (i.e., *physiologically inactive*). Normally free HCl is 0.1%.

(a) **Phloroglucin Test for Free Hydrochloric Acid** (Gunzburg).
See previous page.

(b) **Physiologically Active Hydrochloric Acid**, i.e., free and combined with protein and organic bases (normally about 0.15%).

Willcox's Modified Volhard Method. Two equal quantities (20 ml.) of gastric contents are taken and in one the total chlorides is estimated by adding excess of N/10 silver nitrate and back titrating with ammonium thiocyanate.

From the other quantity free HCl and the acid combined with organic nitrogen compounds are removed by evaporation and gentle ignition, the remaining inorganic chlorides being then determined as before. Difference gives *active* HCl. In gastric ulcer and hyperchlorhydria the active HCl is equal to or nearly equal to the total acidity, and is usually over 0.15%. In gastric carcinoma the active HCl, as found by Willcox, is nearly always much reduced—always under 0.1%. In chronic gastritis the active HCl is often below normal.

Differential Estimation of Physiologically Combined and the Free Acid. The fluid is titrated with alkali in presence of dimethylaminoazobenzene as indicator, the result being the physiologically combined + free hydrochloric acid; then another portion is titrated with alizarin red (1% aqueous solution) as indicator, which gives free hydrochloric acid only. The amount of alkali required in the first titration minus the amount required for the second titration is the amount required by the *physiologically combined hydrochloric acid*, i.e., hydrochloric acid combined with proteins and other weak bases, e.g.:

1st titration showed 0.2% calculated as hydrochloric acid.

2nd titration showed 0.15% free hydrochloric acid.

$0.2\% - 0.15\% = 0.05\%$, physiologically combined hydrochloric acid.

Töpfer's Test. Solution A: 0.5% dimethylaminoazobenzene. Solution B: 1% alcoholic phenolphthalein.

Add one drop of Solution A and one drop of Solution B to 5 ml. of the filtered gastric juice. Titrate with N/10 NaOH until the red colour changes to yellow. This gives the content of free HCl on multiplying the number of ml. of N/10 NaOH by 20 and then by 0.00365.

On titrating the solution with the N/10 soda until a pink colour due to the presence of Solution B is produced, the quantity of N/10 soda used gives the total acidity.

Total chloride concentration and acidity of the gastric contents.—*Brit. chem. Abstr. A.*, 1928, 1153.

III. Organic Acid, Lactic Acid. According to Willcox, great importance should not be attached to presence or absence of this acid. Organic acids in considerable amount are present in carcinoma of the stomach and where much fermentation is going on.

Uffelmann's Test for Lactic Acid (not entirely satisfactory). Ferric chloride solution 1 drop, phenol 0.4 g., water to 50 ml. (Delicacy limit 1: 10,000—the violet colour changes to yellow).

An approximate estimation may be conducted as follows:—

Distil 30 ml. from 40 ml. of the filtered stomach contents the total acidity of which is known. The volatile acids pass over: the residue contains the lactic and hydrochloric acids. The acidity of the distillate (found by titration with N/10 soda, using phenolphthalein as indicator) deducted from the total acidity "A" (found by titrating 10 ml. of the filtered stomach contents in the same manner, the result being expressed in terms of hydrochloric acid) gives the amount of lactic and hydrochloric acids together. If the amount of HCl "H" (found in the same way as "A," but using dimethylaminoazobenzene as indicator) be deducted from this, the remainder is lactic acid.

Mucin. In gastric ulcer and hyperchlorhydria usually absent. In gastric carcinoma a definite precipitate occurs on adding 2% acetic acid. In simple gastritis often present in small amount. It is soluble in sodium hydroxide solution. Dried film is deeply stained reddish violet by thionin staining solution.

Mucus normally is stained faintly, but that met with in chronic gastritis deeply, with methyl green.

Ferment Activity. Determination of pepsin and pepsinogen present is of great importance. The method of Willcox is as follows:—

Action on milk by determination of the activity of the gastric juice by rennin contained (usually proportionate to pepsin) by using a series of tubes containing 5 ml. of milk, to which are added gradually increased quantities of the gastric juice, and the mixtures maintained at 40° for 30 minutes. About 0.2 ml. of normal gastric juice (of the adult) is required in this test.

In gastric carcinoma much more.

In gastric ulcer and hyperchlorhydria usually less (0.05 ml. or less).

In certain cases it may be necessary to estimate renninogen.

Rennin is tested for by adding a few drops of the filtered and neutralised stomach contents to two or three ml. of milk and maintaining the mixture at 98°F. for 15 minutes; resulting coagulation indicates presence.

For testing for **rennin zymogen**, a small quantity of calcium chloride is added prior to incubation. A pocket incubator may be used for these experiments.

Test for the products of **Starch Digestion**. The presence of erythrodeutrin in any quantity (giving a brown colour with Lugol's Solution) one hour after a test breakfast will point to hypochlorhydria.

Gunzburg's Capsule, for testing digestive power, consists of $\frac{1}{8}$ inch of thin rubber tubing, $\frac{1}{4}$ inch in diameter, containing $1\frac{1}{2}$ grains of potassium iodide plugged with pledgets of fibrin at each end.

Fermentation is examined by means of an ordinary Doremus Ureometer.

Estimation of the **digestive power** of the gastric juice is effected with hard boiled egg by examining for peptone after two hours or so at 40°.

Microscopical Examination. This may be necessary in order to interpret the results of chemical examination, e.g., if blood tests are positive.

It is best to use the fasting juice for microscopical examination. The centrifuged deposit is examined for white and red blood cells, epithelial cells, tumour cells, food residues and bacterial organisms. A large number of starch granules and some yeast cells derived from toast will be present after an Ewald meal and oil globules are normally present, being derived from the liquid paraffin used for lubricating the stomach tube.

The presence of erythrocytes, which should be carefully distinguished from oil globules, may indicate true hæmorrhage from an ulcer but may also be present due to bleeding of the gums, trauma by the tube, dilatation of the œsophageal veins as in cirrhosis of the liver or may have oozed from the mucous membrane as in pernicious anæmia.

Leucocytes are normally present in resting juice due to swallowing of saliva. True pus, consisting of clumps of degenerated leucocytes not surrounded by mucus, is commonly found in carcinoma of the stomach.

Squamous epithelial cells are normally present being derived from the mouth and pharynx, but gastric epithelial cells are of pathological significance. Endothelial cells containing phagocytised black particles may be present, being derived from the lungs and should be distinguished from wandering cells with phagocytised blood-pigment from the stomach. In cases of carcinoma and gastritis, isolated gastric epithelial cells may be seen. Tumour cells are rarely seen, but are diagnostic if present.

The presence of food residues such as starch granules, meat fibres, cellulose cell walls of vegetable remains or casein curds in the fasting juice is indicative of pyloric obstruction or carcinoma.

The examination of the bacterial flora is now considered of little value. Sarcinæ are normally present and their absence may indicate carcinoma.

NUTRITION

Knowledge concerning the quantity of food required for health is increasing far less rapidly than knowledge concerning quality. The report on the Physiological Bases of Nutrition drawn up by the Technical Commission of the Health Committee of the League of Nations in 1935 and 1936 remains the most authoritative statement on the amounts of food required at different ages and under different circumstances by human beings. Part I of the Report is quoted in full in the following pages.

ENERGY, PROTEIN AND FAT REQUIREMENTS

All the figures on which the Commission has agreed are average values, and it is essential that they should be interpreted in the light of this fact.

1. **Calorie Requirements.** (a) An adult, male or female living an ordinary everyday life in a temperate climate, and not engaged in manual work, is taken as the basis on which the needs of other age-groups are reckoned. An allowance of 2400 calories net¹ per day is considered adequate to meet the requirements of such an individual. A calorie (i.e., a kilo calorie) is the amount of heat necessary to raise the temperature of 1000 g. of water 1°C. and is sometimes spelt with a capital C.

(b) The following supplements for muscular activity should be added to the basic requirements in (a):²

| | | |
|-----------------|----------------------------|------------------------------|
| Light work: | up to | 75 calories per hour of work |
| Moderate work: | " 75-150 | " " |
| Hard work: | " 150-300 | " " |
| Very hard work: | " 300 calories and upwards | per hour of work |

(c) The energy requirements for other ages and for mothers can be obtained from the following table of coefficients:

| Age (years) | Coefficient | Calories |
|--------------------------|-------------|----------|
| 1- 2 | 0.35 | 840 |
| 2- 3 | 0.42 | 1000 |
| 3- 5 | 0.5 | 1200 |
| 5- 7 | 0.6 | 1440 |
| 7- 9 | 0.7 | 1680 |
| 9-11 | 0.8 | 1920 |
| 11-12 | 0.9 | 2160 |
| 12-15 ³ | 1.0 | 2400 |
| 15 and upwards | 1.0 | 2400 |

¹ The term "net calories" refers to the amount of energy available from the food actually assimilated.

² For statistical purposes, to be comparable with previously adopted standards, 800 calories may be taken as an average supplement for muscular work.

³ The needs of puberty are covered by giving the child of 12-15 years a calorie allowance corresponding to a coefficient of 1, with appropriate supplements for muscular activity and a protein allowance of 2.5 grammes per kilogramme of bodyweight.

The muscular activities characteristic of every healthy child and adolescent necessitate additions to the basic requirements shown in (c). It is suggested that the activities of children of both sexes from 5-11 years be considered as equivalent to light work, of boys from 11-15 years as moderate work, and of girls from 11-15 upwards as light work.

| Women: | | Coefficient | Calories |
|----------|----|-------------|----------|
| Pregnant | .. | 1.0 | 2400 |
| Nursing | .. | 1.25 | 3000 |

Allowance must also be made for women, whether pregnant or not, engaged in household duties; these duties have to be reckoned as equivalent to light work for eight hours daily.

The requirements for babies under 1 year are difficult to specify except in terms of bodyweight; the following allowances are considered adequate:

| Age (months) | | Calories per kilogram of bodyweight |
|--------------|-------|--|
| 0-6 | | 100 |
| 6-12 | | 90 |

2. Protein Requirements. In practice, the protein intake for all adults should not fall below 1 gramme of protein per kilogram of bodyweight. The protein should be derived from a variety of sources, and it is desirable that a part of the protein should be of animal origin.

During growth, pregnancy and lactation, some animal protein is essential, and in the growing period it should form a large proportion of the total protein.

The following allowances of total protein are recommended:

| Age (years) | | Grammes per kilogram of bodyweight |
|--------------------|------------|---------------------------------------|
| 1-3 | | 3.5 |
| 3-5 | | 3.0 |
| 5-12 | | 2.5 |
| 12-15 ¹ | | 2.5 ¹ |
| 15-17 | | 2.0 |
| 17-21 | | 1.5 |
| 21 and upwards | | 1.0 |
| Women: | | |
| Pregnant: | 0-3 months | 1.0 |
| | 4-9 months | 1.5 |
| Nursing | | 2.0 |

3. Fat Requirements. Fat must be a constituent of the normal diet, but the data at present available do not suffice to permit a precise statement of the quantity required. The high content of vitamins A and/or D in certain fats justifies their use in liberal amounts.

¹ See footnote 1, page 713.

4. **The Influence of Climate on Dietary Requirements.** In cold climates, the energy-content of the diet may need to be increased. Where climatic conditions or social customs do not permit of exposure to sunshine, vitamin D should be supplied in the diet.

Proteins. Proteins are the nitrogen-containing substances necessary for building the body tissues. They are very varied in nature and composition and of exceedingly complicated structure. They have high molecular weights, e.g., that of casein is 33,600, of ox hæmoglobin 34,300, of egg albumin, 36,000. On hydrolysis by boiling with acids or alkalis or by the action of enzymes, they yield amino-acids. About twenty-two different amino-acids are found in most proteins. Six others have been found only in special proteins and several others have been described and are claimed to have been isolated by certain workers. The various amino-acids are combined in different proportions and in different ways in different proteins. Thus, theoretically, an almost infinitely large number of proteins of different composition may exist, but actually the number so far recognised is relatively small. The "same" proteins in different species of animals may in fact be different, but the differences cannot be detected by chemical or physical methods. Only by sensitising an animal to a protein (e.g., hen's egg albumin) and then injecting another sample of ovalbumin into it, can it be determined whether the second sample is really of the same composition as the first. If it is the same, the animal will have an anaphylactic shock; if it is different, the animal will make no response.

Proteins are broken down in the animal body into proteose and peptones in the stomach and into amino-acids in the intestines. These comparatively simple substances are absorbed into the bloodstream and from there taken out as required to build up fresh tissue in place of that which has been broken down. The growing child needs more protein per kilo of bodyweight than the adult, for he has also to increase his amount of nitrogen-containing tissues. Up to the beginning of this century only thirteen amino-acids had been discovered and there was very little information on their quantitative distribution. During the last forty years however, methods of analysis, both quantitative and qualitative, have been developed by means of which the composition of a protein can be fairly well determined. By feeding experiments on animals the importance of different amino-acids in nutrition has been estimated. Thus it has been shown that gliadin, one of the proteins of wheat, is deficient in lysine; zein, the protein of maize, is deficient in lysine and tryptophane, and gelatin is deficient in cystine, valine, isoleucine, tyrosine, tryptophane and hydroxyglutamic acid. Table I (taken from *Physiological Reviews*, 1938, 18, 109. *Nutritive Significance of Amino-acids* by W. C. Rose) shows which amino-acids are necessary for the growth of the young rat.

Table I

| INDISPENSABLE | DISPENSABLE |
|---------------|----------------------|
| Lysine | Glycine |
| Tryptophane | Alanine |
| Histidine | Serine |
| Phenylalanine | Norleucine |
| Leucine | Aspartic acid |
| Isoleucine | Glutamic acid |
| Threonine | Hydroxyglutamic acid |
| Methionine | Proline |
| Valine | Hydroxyproline |
| *Arginine | Citrulline |
| | Cystine |

* Arginine can be synthesised by the animal organism, but not at a sufficiently rapid rate to meet the demands of *normal* growth.

It should be noted that an incomplete protein can be "made good" by supplementing it with another protein which contains adequate amounts of the missing amino-acid. It is not necessary to add the amino-acid alone. Increasing knowledge of the composition of proteins and of which amino-acids are necessary for the nutrition and growth of animals has led to the adoption of the term "biological value of a protein." A protein of high biological value contains all the necessary amino-acids in adequate amounts; one of low biological value does not. Animal proteins, with the exception of gelatin, have a high biological value, vegetable proteins have a low biological value. That is why it is so necessary to include some animal protein (milk, eggs, cheese, or meat) in the diet each day.

References to Proteins

The nutritive significance of amino-acids.—W. C. Rose, *Physiol. Rev.*, 1938, 18, 109.

Planned war-time nutrition. The place of protein in the diet.—J. C. Drummond, *Brit. med. J.*, 1/1942, 21.

The chemistry of flesh foods and their losses on cooking.—R. A. McCance and H. L. Shipp, *Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 187, 1933.

Quality and quantity of protein in relation to human health and disease.—D. P. Cuthbertson, *Nutr. Abstr. Rev.*, 1940, 10, 1.

Determination of the protein requirements of man.—I. Leitch and J. Duckworth, *Nutr. Abstr. Rev.*, 1937, 7, 257.

Fats. Fats are a less bulky source of energy than carbohydrates. Their chief importance in the diet is as carriers of the fat-soluble vitamins. Hence it is desirable that vitamin-containing fats should be used in diets as much as possible, and particularly in the diet of children whose fat-tolerance is less than that of adults. Fats are converted into glycerol and fatty acids by the lipase of the pancreatic juice in the intestine. Bile emulsifies the fats and thereby facilitates the action of the lipase. Certain of the unsaturated fatty

acids, linoleic and linolenic, have been shown to be necessary to the rat to prevent a condition known as "scaley tail." No evidence is available to show that these are beneficial to human beings; in fact it would be extremely difficult to feed human beings on a diet that did not contain ample supplies of these fatty acids. Claims that certain face creams containing these substances have had remarkably good effects cannot be considered seriously. About 70% of the food fat is absorbed through the lacteals. The rest passes directly into the capillaries in the walls of the intestine and thence by the portal vein to the liver. Absorption is probably greatly facilitated by the bile acids which, passing into the bloodstream with the fats, are withdrawn again by the liver and re-secreted in the bile. It is now generally accepted that fat in the diet exerts a sparing action on vitamin B₁, i.e., if an animal is fed on a diet containing fat, it requires less vitamin B₁. The explanation of this is probably that vitamin B₁ is necessary for the formation of fat from carbohydrate through the intermediary pyruvic acid and if fat replaces carbohydrate in the diet, less is synthesised in the animal body. Many of the unsaponifiable constituents of naturally occurring fats are probably of nutritional importance. Vitamin A may occur as the alcohol or as an ester. Vitamin D probably occurs only as a sterol (or as several sterols). Kephalins and lecithins may be intermediaries in fat metabolism. Choline, a constituent part of the molecule of lecithin and other phospholipids, has lipotropic activity, i.e., it helps in the withdrawal of fat from the liver and prevents the development of fatty livers. Synthetic homocholine has been found to be more effective than choline. The amino-acid methionine is also lipotropic but cystine has the opposite effect.

The influence of choline on animal metabolism.—*Brit. med. J.*, ii/1941, 585.

Carbohydrates. Carbohydrates supply energy to the body by their oxidation. They are classified in three groups:—

- (a) Monosaccharides, e.g., glucose, fructose, galactose, etc.
- (b) Disaccharides, e.g., sucrose, maltose, lactose, etc.
- (c) Polysaccharides, e.g., starch, cellulose, glycogen, etc.

The Monosaccharides require no digestion before absorption. Different monosaccharides are absorbed at different rates. Galactose is the most easily absorbed, glucose nearly as easily, and then come fructose, mannose, xylose and arabinose in decreasing ease of absorbability. This was determined by experiments on rats and dogs. When two or more sugars are mixed or when a mixed meal has been taken the rate of absorption of a sugar is very much slower than when the sugar is taken in pure solution.

The Disaccharides are hydrolysed into their constituent monosaccharides before absorption. This is effected by the invertase of the pancreatic juice. Sucrose is converted into glucose and fructose, maltose into glucose, and lactose into glucose and galactose.

The Polysaccharides, such as starch and dextrin, are hydrolysed by amylolytic enzymes into maltose which is hydrolysed into glucose. Cellulose is not hydrolysed in the human digestive tract and is not absorbed. Ruminants however can digest cellulose by the action of bacteria present in the gut.

The ptyalin of saliva has very little time to act on starches in the mouth but probably does so to a certain extent in the interior of the mass of food in the stomach before the pH at which it is most active is changed by the gastric juice which slowly permeates the mass. The theory that the cause of caries is the action on the enamel of acids formed by the fermentation of sugars in the mouth has been criticised from many practical stand-points. The influence of vitamin D on the formation of strong enamel is becoming widely recognised and whatever the cause of caries may prove to be it is obvious that the stronger the enamel the longer will it take for the offensive action to become dangerous.

Inorganic Salts. Table 2 has been taken from the *Composition of Optimal and Marginal Diets* by Bacharach and Drummond, *Chem. & Ind.*, 1940, 59, 37, and shows the daily requirements, for a man of 10 stone weight, of the elements occurring in inorganic salts.

Table 2

| | MARGINAL DIET | OPTIMAL DIET |
|-------------------|---------------|----------------|
| NON-METALS | | |
| Phosphorus | 1.0 g. | 1.5 g. |
| Chlorine | 7.0 g. (?) | 7.0 g. |
| Iodine | 0.05 mg. (?) | 0.05 mg. |
| METALS | | |
| Sodium | 5.0 g. (?) | 5.0 g. (?) |
| Potassium | 3.5 g. (?) | 3.5 g. (?) |
| Calcium | 0.75 g. | 1.5 g. |
| Magnesium | + ? | 0.5 g. (?) |
| Iron | 10.0 mg. | 20.0 mg. |
| Copper | + ? (inf.) | 3.0 mg. (inf.) |
| Cobalt | + (inf.) | + (inf.) |
| Manganese | ? (inf.) | ? (inf.) |
| Zinc | ? ? (inf.) | ? ? (inf.) |

inf. = argument by inference, e.g., from experiments on animals:
+ = required, but quantity not known: ? = probably required.

It is evident from the table above that there is very little exact knowledge as to which elements are needed in the diet or in what amounts.

Calcium deficiency results in badly formed bones and teeth. Even an abundance of vitamin D cannot make up for a lack of either calcium or phosphorus though it can rectify a harmful ratio of the Ca/P in the diet. Even if there is enough of both elements, calcification will be poor unless the faulty ratio is rectified by vitamin D. A deficiency of calcium also results in decreased growth rate and premature death. Milk and green vegetables are the richest sources of calcium, but certain vegetables, e.g., spinach, contain oxalic acid which precipitates the calcium in an insoluble and unavailable form.

Cobalt has been shown to be necessary for sheep and cattle. A deficiency results in a blood disease known in different parts of the world as "Denmark disease," "coast disease," the "pines," "bush sickness," and "salt sick."

It is stated that the quantity of cobalt required to cure affected sheep is 1 mg. daily for 14 days (H. H. Corner, *Brit. med. J.*, ii/1939, 169).

Copper is necessary also (in traces) for the formation of hæmoglobin, though it does not form any part of the hæmoglobin molecule. Many oxidases are Cu-protein compounds.

Iodine. The development of goitre in inhabitants of districts where iodine is scarce is well known, as also is the striking effect of adding iodine to the drinking water or to the table salt in such districts. Mellanby distinguishes between simple and toxic goitres though it may be impossible to distinguish between the two by examining their microscopic structures. In "Nutrition and Diseases" (1934), he says "The simple goitre is due primarily to a deficiency of iodine in the body whereas the toxic goitre is caused mainly by some mechanism pulling out the colloid with its active principle as soon as it is formed into the general circulation and thus producing a local absence of iodine in the gland itself, and excess in the blood. Both mechanisms produce thyroid hyperplasia." The simple form can be brought back to the resting stage by iodine therapy. The typical form associated with hyperthyroidism can be brought partially back to the resting stage and kept there by continuous treatment. Occasionally after months or years complete involution is effected. "Cessation of iodine administration at any stage short of complete involution of the gland is associated with dispersal of the accumulated colloid and exacerbation of symptoms of hyperthyroidism which may be very severe."

Iron is necessary for hæmoglobin formation. When it is deficient in the food, anæmia develops. Whole milk is deficient in iron but the new-born infant has large reserves in its liver. Not all iron present in foodstuffs is in an available form, and it has been claimed lately that more iron is assimilable from foods when ascorbic acid is taken also. Iron tonics have not been effective until foods containing vitamin C were taken.

Magnesium. When chlorine, potassium or sodium is withheld from the diet, excretion of these elements is reduced to a minimum and it may be several weeks before any symptoms of deficiency occur. On the other hand, when the amount of magnesium in the diet of young rats is reduced to 1·8 parts per million, in 3 to 5 days they develop a condition of hyperæmia of the skin which increases up to the eleventh or fourteenth day when it begins to subside and is followed by pallor and finally by cyanosis. The rats show great irritability and by the eighteenth day any sudden disturbance startles the rats so much that they have convulsive seizures and eighty per cent. of them die.

Manganese deficiency in the food of rats resulted in an inability of the mothers to suckle their young and an apparent lack of all maternal instinct. In the male rats there was degeneration of the testicles. In chickens this deficiency produced "slipped tendon" and eggs of low hatchability.

Zinc is necessary for the growth of young rats and may be a factor in the beri-beri syndrome, for Eggleton has found that the Zn content of the epidermal structure of beri-beri patients was but half that of healthy persons.

Other elements which are generally found in human tissues are arsenic, aluminium, rubidium, bromine, fluorine, silicon, barium and nickel, but none of them has been found to be essential for health and growth. Fluorine is harmful if more than a trace is present in the diet. Water containing more than 1 part of fluorine per million produces mottled teeth in children, but increased immunity to dental caries is associated with increased fluorine intake. The toxicity of aluminium was reviewed by Burn who estimated that the daily intake of this element by a human being might be as much as 13 mg., though ten times this amount had no ill effects. Beal *et al.*, by cooking food in glass and aluminium utensils respectively, showed that the amounts of aluminium taken up by neutral foods was negligible, and that the amounts taken up by acid foods or by foods cooked with bicarbonate of soda were larger but not serious.

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COMPOSITION AND CALORIE VALUE OF FOODS

One gramme of either protein or carbohydrate yields on combustion 4.1 calories of heat. One gramme of fat yields on an average 9.3 calories, and one gramme of alcohol yields 7.0 calories.

To calculate the calorie value of any portion of diet, find the weights of protein, fat and carbohydrate in the portion from the percentage composition of the food, then multiply the weights of proteins and carbohydrate by 4.1 and the weight of fat by 9.3. The sum of these products is the number of calories available from the portion of food. The following figures have been selected from *The Chemical Composition of Foods* by R. A. McCance and E. M. Widdowson (*Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 235, 1940).

| Food | g. per 100 g. | | | Calories per 100 g. | mg. per 100 g. | |
|--------------------------------|--------------------------|------|--|---------------------------|----------------|------|
| | Protein (N X 6.25) | Fat | Avail- able carbo- hydrate (as glucose) | | Ca | Fe |
| Cereals and Cereal Food | | | | | | |
| Arrowroot | 0.4 | 0.1 | 90.6 | 374 | 7.0 | 1.95 |
| Barley, pearl, boiled .. | 2.9 | 0.6 | 27.6 | 130 | 3.4 | 0.23 |
| Biscuits, cream crackers | 9.3 | 33.0 | 57.5 | 579 | 17.9 | 0.96 |
| " digestive | 10.5 | 20.5 | 66.0 | 505 | 43.6 | 1.57 |
| Bread, white | 7.9 | 0.7 | 53.7 | 260 | 23.1 | 1.0 |
| " wholemeal | 8.4 | 1.6 | 43.8 | 229 | 30.0 | 2.70 |
| Macaroni, boiled | 3.6 | 0.6 | 23.7 | 119 | 8.1 | 0.45 |
| Oatmeal, porridge | 1.5 | 0.9 | 8.2 | 48 | 6.3 | 0.47 |
| Rice, polished, boiled | 2.3 | 0.3 | 29.6 | 133 | 1.3 | 0.16 |
| Sago (dry) | 0.3 | 0.2 | 94.0 | 389 | 9.8 | 1.18 |
| Tapioca (dry) | 0.4 | 0.1 | 95.0 | 392 | 8.2 | 0.32 |
| Dairy Products | | | | | | |
| Butter, fresh | 0.4 | 85.1 | Tr. | 793 | 14.8 | 0.16 |
| Cheese, Cheddar | 24.9 | 34.5 | Tr. | 423 | 810.0 | 0.57 |
| Cream | 1.8 | 42.0 | 2.3 | 407 | 59.2 | 0.23 |
| Egg white | 9.0 | Tr. | 0.0 | 37 | 5.2 | 0.10 |
| " yolk | 16.2 | 30.5 | 0.0 | 350 | 131.5 | 6.13 |
| Milk, fresh, whole | 3.3 | 3.7 | 4.6 | 67 | 120.0 | 0.08 |
| " " skimmed | 3.4 | 0.2 | 4.8 | 36 | 124.0 | 0.08 |

| Food | g. per 100 g. | | | Calories per 100 g. | mg. per 100 g. | |
|---|--------------------------|------|--|---------------------------|----------------|------|
| | Protein (N x 6.25) | Fat | Avail- able carbo- hydrate (as glucose) | | Ca | Fe |
| Meat, Poultry and Game | | | | | | |
| Bacon, streaky, fried .. | 24.0 | 46.0 | 0.0 | 526 | 52.3 | 3.2 |
| Beef, sirloin, roast, lean and fat. . . | 21.3 | 32.1 | 0.0 | 385 | 5.8 | 4.6 |
| Chicken, roast .. | 29.6 | 7.3 | 0.0 | 189 | 14.5 | 2.6 |
| " boiled .. | 26.2 | 10.3 | 0.0 | 203 | 10.7 | 2.1 |
| Ham, boiled, lean and fat .. | 16.3 | 39.6 | 0.0 | 435 | 12.7 | 2.5 |
| Kidney, sheep, fried .. | 28.0 | 9.1 | 0.0 | 199 | 16.6 | 14.5 |
| " ox, stewed .. | 25.7 | 5.8 | 0.0 | 159 | 20.8 | 7.1 |
| Liver, calf, fried .. | 29.0 | 14.5 | 2.4 | 263 | 8.8 | 21.7 |
| Mutton, leg, boiled .. | 25.8 | 16.6 | 0.0 | 260 | 3.6 | 5.1 |
| Pheasant, roast .. | 30.8 | 9.3 | 0.0 | 213 | 49.3 | 8.4 |
| Pork, loin, lean and fat .. | 19.5 | 40.4 | 0.0 | 455 | 7.5 | 2.3 |
| Rabbit, stewed .. | 26.6 | 7.7 | 0.0 | 180 | 11.3 | 1.9 |
| Sweetbreads, stewed .. | 22.7 | 9.1 | 0.0 | 178 | 14.3 | 1.6 |
| Tongue, ox, pickled .. | 19.1 | 23.9 | 2.3 | 310 | 30.9 | 3.0 |
| Veal, fillet, roast .. | 30.5 | 11.5 | 0.0 | 232 | 14.3 | 2.5 |
| Fish | | | | | | |
| Bloaters, grilled .. | 22.6 | 17.4 | 0.0 | 255 | 123.0 | 2.2 |
| Cod, steamed .. | 18.0 | 0.9 | 0.0 | 82 | 14.6 | 0.5 |
| Haddock, fresh steamed | 22.0 | 0.8 | 0.0 | 97 | 54.6 | 0.7 |
| Hake, steamed .. | 18.5 | 3.3 | 0.0 | 107 | 15.9 | 0.6 |
| Halibut, steamed .. | 22.7 | 4.0 | 0.0 | 130 | 13.0 | 0.6 |
| Herring, fried .. | 21.8 | 15.1 | 1.5 | 236 | 38.6 | 1.9 |
| " roe, fried .. | 23.4 | 15.8 | 4.7 | 262 | 15.7 | 1.5 |
| Kippers, baked .. | 23.2 | 11.4 | 0.0 | 201 | 64.8 | 1.4 |
| Mackerel, fried .. | 20.0 | 11.3 | 0.0 | 187 | 28.4 | 1.2 |
| Oysters, raw .. | 10.2 | 0.9 | Tr. | 50 | 186.0 | 6.0 |
| Plaice, steamed .. | 18.1 | 1.9 | 0.0 | 92 | 37.7 | 0.6 |
| Salmon, fresh steamed | 19.1 | 13.0 | 0.0 | 199 | 28.9 | 0.8 |
| " tinned .. | 19.7 | 6.0 | 0.0 | 137 | 66.4 | 1.3 |
| Sardines, tinned .. | 20.4 | 22.6 | 0.0 | 294 | 409.0 | 4.0 |
| Shrimps .. | 22.3 | 2.4 | 0.0 | 114 | 320.0 | 1.8 |
| Sprats, fresh, fried .. | 22.3 | 37.9 | 0.0 | 444 | 707.0 | 4.5 |
| Trout, steamed .. | 22.3 | 4.5 | 0.0 | 133 | 35.8 | 1.0 |
| Fruit | | | | | | |
| Apples, English, eating | 0.3 | Tr. | 11.7 | 49 | 3.5 | 0.29 |
| " cooking, stewed | 0.1 | Tr. | 4.4 | 19 | 1.7 | 0.13 |
| Apricots, dried, stewed, without sugar .. | 2.0 | Tr. | 18.0 | 82 | 38.4 | 1.70 |
| Bananas .. | 1.1 | Tr. | 19.2 | 83 | 6.8 | 0.41 |
| Blackberries, raw .. | 1.3 | Tr. | 6.4 | 32 | 63.3 | 0.85 |
| Cherries .. | 0.6 | Tr. | 11.9 | 51 | 15.9 | 0.38 |
| Currants, black, stewed, without sugar .. | 0.6 | Tr. | 4.6 | 22 | 42.1 | 0.89 |
| Damsons, stewed, with- out sugar .. | 0.3 | Tr. | 6.6 | 28 | 16.2 | 0.28 |
| Dates .. | 2.0 | Tr. | 63.9 | 270 | 67.9 | 1.61 |
| Gooseberries, green, stewed, without sugar .. | 0.6 | Tr. | 1.7 | 9 | 14.2 | 0.16 |
| Grapes, black .. | 0.6 | Tr. | 15.5 | 66 | 4.2 | 0.34 |

| Food | g. per 100 g. | | | Calories per 100 g. | mg. per 100 g. | |
|---|---------------------------------|------|--|---------------------------|----------------|------|
| | Protein (N \times 6.25) | Fat | Avail- able carbo- hydrate (as glucose) | | Ca | Fe |
| Fruit (continued) | | | | | | |
| Grapefruit | 0.6 | Tr. | 5.3 | 24 | 17.1 | 0.26 |
| Lemons, whole .. | 0.8 | Tr. | 3.2 | 16 | 107.0 | 0.35 |
| Melons, cantaloupe .. | 1.0 | Tr. | 5.3 | 26 | 19.1 | 0.81 |
| Oranges | 0.8 | Tr. | 8.5 | 38 | 41.3 | 0.33 |
| Peaches | 0.6 | Tr. | 9.1 | 40 | 4.8 | 0.38 |
| Pears, English, eating .. | 0.2 | Tr. | 10.4 | 43 | 6.9 | 0.22 |
| Pineapple, tinned in syrup | 0.3 | Tr. | 16.5 | 69 | 13.4 | 1.70 |
| Plums, Victoria dessert | 0.6 | Tr. | 9.6 | 42 | 11.0 | 0.36 |
| Prunes, stewed, with- out sugar | 0.9 | Tr. | 15.5 | 67 | 14.5 | 1.12 |
| Raisins, dried | 1.1 | Tr. | 64.4 | 269 | 60.6 | 1.55 |
| Raspberries, raw | 0.9 | Tr. | 5.6 | 27 | 40.7 | 1.21 |
| Rhubarb, stewed, with- out sugar | 0.4 | Tr. | 0.7 | 5 | 72.0 | 0.28 |
| Strawberries | 0.6 | Tr. | 6.2 | 28 | 22.0 | 0.71 |
| Sultanas, dried | 1.7 | Tr. | 64.7 | 272 | 52.2 | 1.82 |
| Nuts | | | | | | |
| Almonds | 20.5 | 53.5 | 4.3 | 600 | 247.0 | 4.23 |
| Barcelona nuts | 12.9 | 64.0 | 5.2 | 669 | 170.0 | 2.97 |
| Brazil nuts | 13.8 | 61.5 | 4.1 | 645 | 178.0 | 2.82 |
| Chestnuts | 2.3 | 2.7 | 36.6 | 185 | 46.0 | 0.89 |
| Cob nuts | 9.0 | 36.0 | 6.8 | 400 | 44.0 | 1.06 |
| Coconut, fresh | 3.8 | 36.0 | 3.7 | 366 | 13.0 | 2.08 |
| Peanuts | 28.1 | 49.0 | 8.6 | 606 | 61.0 | 2.04 |
| Walnuts | 12.5 | 51.5 | 5.0 | 551 | 61.0 | 2.35 |
| Vegetables | | | | | | |
| Asparagus, boiled (weighed as served) | 1.7 | Tr. | 0.6 | 9 | 12.9 | 0.45 |
| Beans, baked | 6.0 | 0.4 | 17.3 | 99 | 61.6 | 2.05 |
| " butter, boiled .. | 7.1 | Tr. | 17.1 | 99 | 18.7 | 1.67 |
| " runner, boiled .. | 0.8 | Tr. | 0.9 | 7 | 25.6 | 0.59 |
| Beetroot, boiled | 1.8 | Tr. | 9.9 | 48 | 30.0 | 0.70 |
| Brussels sprouts, boiled | 2.4 | Tr. | 1.7 | 17 | 27.1 | 0.63 |
| Cabbage, Savoy, boiled | 1.3 | Tr. | 1.1 | 10 | 52.5 | 0.72 |
| Carrots, old, boiled .. | 0.6 | Tr. | 4.3 | 20 | 36.9 | 0.37 |
| Cauliflower, boiled .. | 1.5 | Tr. | 1.2 | 11 | 23.0 | 0.48 |
| Celery, raw | 0.9 | Tr. | 1.3 | 9 | 52.2 | 0.61 |
| Cucumber, raw | 0.6 | Tr. | 1.8 | 10 | 22.8 | 0.30 |
| Leeks, boiled | 1.8 | Tr. | 4.6 | 26 | 60.5 | 2.00 |
| Lettuce, raw | 1.1 | Tr. | 1.8 | 12 | 25.9 | 0.73 |
| Marrow, boiled | 0.4 | Tr. | 1.4 | 7 | 13.6 | 0.22 |
| Onions, raw | 0.9 | Tr. | 5.2 | 25 | 31.2 | 0.30 |
| " boiled | 0.6 | Tr. | 2.7 | 14 | 24.4 | 0.25 |
| " fried | 1.8 | 33.3 | 10.1 | 359 | 61.0 | 0.59 |
| Parsnips, boiled | 1.3 | Tr. | 13.5 | 61 | 35.5 | 0.45 |
| Peas, fresh, boiled .. | 5.0 | Tr. | 7.7 | 52 | 12.6 | 1.22 |
| Potatoes, old, boiled .. | 1.4 | Tr. | 19.7 | 87 | 4.3 | 0.48 |
| Radishes | 1.0 | Tr. | 2.8 | 16 | 43.7 | 1.88 |
| Spinach, boiled | 5.1 | Tr. | 1.4 | 27 | 595.0 | 4.00 |
| Swedes, boiled | 0.9 | Tr. | 3.8 | 19 | 41.5 | 0.29 |
| Tomatoes, raw | 0.9 | Tr. | 2.8 | 15 | 13.3 | 0.43 |
| Turnips, boiled | 0.7 | Tr. | 2.3 | 12 | 55.0 | 0.35 |
| Watercress, raw | 2.9 | Tr. | 0.7 | 15 | 222.0 | 1.62 |

| Food | g. per 100 g. | | | Calories per 100 g. | mg. per 100 g. | |
|---|--------------------------|------|--|---------------------------|----------------|------|
| | Protein (N × 6.25) | Fat | Avail- able carbo- hydrate (as glucose) | | Ca | Fe |
| Sugars, Preserves and Sweetmeats | | | | | | |
| Chocolate, milk .. | 7.4 | 34.1 | 52.3 | 561 | 175.0 | 1.67 |
| " plain .. | 4.6 | 32.5 | 58.7 | 562 | 26.1 | 3.28 |
| Chutney, tomato .. | 1.1 | 0.1 | 37.2 | 158 | 26.2 | 0.93 |
| Honey, in jars .. | 0.4 | Tr. | 76.4 | 315 | 5.3 | 0.39 |
| Ice cream .. | 3.9 | 13.2 | 17.5 | 211 | 152.0 | 0.21 |
| Jam, fruit with edible seeds .. | 0.6 | 0.0 | 69.0 | 285 | 24.2 | 1.47 |
| Jam, stone fruit .. | 0.4 | 0.0 | 69.3 | 285 | 12.0 | 1.02 |
| Marmalade .. | 0.1 | 0.0 | 69.5 | 285 | 34.7 | 0.58 |
| Syrup, golden .. | 0.3 | 0.0 | 79.0 | 325 | 26.4 | 1.45 |
| Toffee, home-made .. | 0.2 | 6.2 | 87.8 | 418 | 11.0 | 0.55 |
| Beverages | | | | | | |
| Bournvita, dry .. | 11.4 | 7.5 | 67.6 | 394 | 89.0 | 3.3 |
| Bovril .. | 29.0 | 0.7 | 0.0 | 125 | 52.0 | 12.1 |
| Cocoa powder .. | 20.4 | 25.6 | 35.0 | 464 | 51.2 | 14.3 |
| Coffee, ground, roasted | 12.5 | 15.4 | 28.5 | 311 | 133.0 | 4.1 |
| Malted milk, Horlick's | 14.4 | 8.6 | 70.8 | 430 | 272.0 | 1.3 |
| Marmite .. | 10.0 | Tr. | 0.0 | 41 | 77.3 | 5.2 |
| Ovaltine .. | 13.2 | 7.9 | 61.6 | 378 | 339.0 | 3.5 |
| Oxo, cubes .. | 31.9 | 3.8 | 0.0 | 166 | 101.5 | 14.0 |
| Tea, Indian, infusion | 0.1 | 0.0 | 0.0 | <1 | 0.3 | Tr. |
| Virol .. | 4.6 | 12.8 | 59.6 | 383 | 108.0 | 17.6 |
| Beers, etc. | | | | | | |
| Pale ale, bottled .. | 0.2 | Tr. | 3.0 | 56* | 13.6 | 0.07 |
| Mild ale, bottled .. | 0.3 | Tr. | 3.7 | 50* | 12.7 | 0.08 |
| Strong ale .. | 0.5 | Tr. | 4.9 | 78* | 16.9 | 0.10 |
| Stout .. | 0.4 | Tr. | 4.1 | 51* | 10.3 | 0.14 |

* Including calories yielded by alcohol (= 7 cal. per g.).

Tr. in the above columns = Trace.

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- Determination of the caloric requirements of man.—J. B. Orr and I. Leitch, *Nutr. Abstr. Rev.*, 1937, 509.
- Trends in nutrition.—Sir J. Orr, *Brit. med. J.*, i/1941, 73.

VITAMINS

The number of vitamins thought to exist is increasing. Some, but not all of the newly discovered ones have been shown to be necessary to human beings. The chemical composition of vitamins A, B₁, C, D₂, D₃, E, K, riboflavin, nicotinic acid, pyridoxine and pantothenic acid is known and some of them have been synthesised. On the other hand, "vitamin" B₆ and "factor" Y are now thought to be identical with pyridoxine. Fresh evidence of the existence of the "casein factor" has come from America but the factor has not yet been isolated.

The first International Conference on the Standardisation of the Vitamins was held in June 1931. A second conference was held in June 1934, when the use which had been made of the standards since the first conference was discussed. Certain changes in the standards were made in accordance with the increased knowledge of the chemistry of the vitamins but the actual weight of a unit of each standard was adjusted so that the biological value of each unit remained unchanged. Plans for a third meeting of the Conference to be held in 1939 had to be abandoned owing to the outbreak of war.

The Determination of Vitamins

Vitamin A in liver oils and concentrates can be determined by physical methods, though there is still a lack of agreement between American and British workers as to the "conversion factor" to be used for converting the spectroscopic determination into the vitamin A value. The spectroscopic determination of vitamin A in butters and margarines is still uncertain. Vitamin B₁ in pure solution can be determined by physical methods, but there is some disagreement as to the best method of extracting this factor from foodstuffs for its determination. Vitamin C in fruit juices can be determined chemically, but various modifications of the procedure have been advocated for its determination in urine and other body fluids and tissue extracts. Vitamin D must still be determined biologically even in liver oils and concentrates. Vitamin E in concentrates and pure solutions can be determined chemically. Work on the chemical determination of riboflavin, nicotinic acid, pyridoxine, and pantothenic acid is in progress. The proof that a chemical or physical method of determination is reliable depends on its agreement with biological determinations.

A biological determination involves the simultaneous testing of the standard of reference, for animal reactions fluctuate from time to time to such an extent that no animal reaction at any time can be taken as an absolute measure of the activity of the dose given. It is only relative to the activity of the dose of standard tested simultaneously.

The Permanent Commission on Biological Standardisation of the Health Organisation of the League of Nations have adopted the following units for international use:—

(1) **Vitamin A.** The growth-promoting activity for rats of 0.6 μ g. of standard β -carotene.

(2) **Vitamin B₁.** The vitamin B₁ activity of 3 µg. of pure synthetic vitamin B₁ hydrochloride.

(3) **Vitamin C.** The anti-scorbutic activity of 0.05 milligram of standard *l*-ascorbic acid.

(4) **Vitamin D.** The anti-rachitic activity of 1 milligram of standard solution of irradiated ergosterol, or of 0.025 µg. of crystalline calciferol, or of activated 7-dehydro-cholesterol.

(5) **Vitamin E.** The vitamin E activity of 1 milligram of standard synthetic, racemic α -tocopheryl acetate (representing the average value for the total median fertility dose preventing resorption-gestation in rats deprived of vitamin E).

All the International Standard preparations of vitamins are kept at the National Institute for Medical Research, London.

The Biological Standardisation of Vitamins.—K. H. Coward, *Nutr. Abstr. Rev.*, 1934-5, 705.

An account of work carried out by many workers in preparation for the second International Conference on Vitamin Standardisation, 1934.—E. M. Hume and H. Chick, *Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 202, 1935.

The adoption of crystalline vitamin B₁ hydrochloride as the new international standard of vitamin B₁ and comparison of its potency with that of the former standard. A summary of co-operative experiments organised by the Accessory Food Factors Committee.—*Bull. Hlth Org. L. o. N.*, 1940-1, 9, 371.

Other Units for Vitamin Activity Still in Use. Various units of vitamin activity were used before the International Standards of Reference became available. These units are still being used by some workers at the present day, and "factors" are used for converting into international units, the results expressed in these units. The U.S.P. Revision Committee has suggested some of these factors, not with the purpose of encouraging the use of the old units but in order to give approximate values in international units of stocks of oils, already on the market, whose potencies have been stated in the old units. The U.S.P. Commission realises (as indeed do most other workers) that the old units were defined as a certain amount of animal reaction which itself varied from time to time and in different laboratories according to uncontrollable conditions. This means that an oil assayed at 100 "units" of vitamin D at one time might easily give a result of 200 "units" at another time or in another worker's laboratory. In contrast to this, the international units are particular weights of particular preparations of the vitamins. Thus an estimation of the vitamin content of a substance made by comparing the effect of a dose of that substance with the effect of a dose of a standard at the same time on similar animals is independent of the sensitivity of the rats at the time that the test is made. Within the limits of experimental error the same estimation of the potency of an oil should be obtained by different workers regardless of the details of the technique employed.

The Accuracy Obtainable in Biological Tests for Vitamins. The "error" of vitamin tests is summarised in the following table. (*B.P. Comm., Report of the Sub-Committee on the Accuracy of Biological Assays.*)

Limits of Error
with varying numbers of animals

Vitamin A

| | (a) 3 weeks' test | | (b) 5 weeks' test | |
|---------------|-----------------------|-----------------------|-----------------------|-----------------------|
| | P = 0.99 per cent. | P = 0.95 per cent. | P = 0.99 per cent. | P = 0.95 per cent. |
| 20 rats | 30 and 339 | 40 and 253 | 37 and 272 | 47 and 214 |
| 40 rats | 42 and 237 | 52 and 193 | 49 and 203 | 58 and 171 |
| 80 rats | 54 and 184 | 63 and 159 | 61 and 165 | 68 and 146 |

Vitamin B₁

| | (a) Pigeons | | (b) Rats | | |
|------------|-----------------------|-----------------------|------------|-----------------------|-----------------------|
| | P = 0.99 per cent. | P = 0.95 per cent. | | P = 0.99 per cent. | P = 0.95 per cent. |
| 20 pigeons | 15 and 652 | 24 and 417 | 10 rats .. | 65 and 154 | 72 and 139 |
| 40 pigeons | 27 and 377 | 36 and 274 | 20 rats .. | 74 and 135 | 79 and 126 |
| 80 pigeons | 39 and 255 | 49 and 204 | 40 rats .. | 81 and 124 | 85 and 118 |

Vitamin C

| | | (a) Teeth | | (b) Growth (of rats) | |
|----------------|---------|-----------------------|-----------------------|---|-----------------------|
| | | P = 0.99 per cent. | P = 0.95 per cent. | P = 0.99 per cent. | P = 0.95 per cent. |
| 20 guinea pigs | y = 3 | 51 and 149 | 63 and 137 | 82 and 139 | 86 and 126 |
| 40 guinea pigs | | 65 and 135 | 74 and 126 | 86 and 124 | 89 and 117 |
| 80 guinea pigs | | 76 and 124 | 81 and 119 | 90 and 115 | 92 and 111 |
| 20 guinea pigs | y = 2.5 | 36 and 164 | 51 and 149 | At a level of dosage just sufficient to main- tain weight for six weeks. | |
| 40 guinea pigs | | 55 and 145 | 66 and 134 | | |
| 80 guinea pigs | | 68 and 132 | 76 and 124 | | |

y = the amount of protection ranging from 0 to 4.

Vitamin D

| | (a) X-ray | | (b) Ash content of bone | | (c) Line test | |
|------------|-----------------------|-----------------------|----------------------------|-----------------------|-----------------------|-----------------------|
| | P = 0.99 per cent. | P = 0.95 per cent. | P = 0.99 per cent. | P = 0.95 per cent. | P = 0.99 per cent. | P = 0.95 per cent. |
| 20 rats .. | 63 and 159 | 70 and 142 | 59 and 170 | 67 and 150 | 49 and 215 | 59 and 176 |
| 40 rats .. | 72 and 139 | 78 and 128 | 69 and 146 | 75 and 133 | 61 and 168 | 68 and 146 |
| 80 rats .. | 79 and 126 | 84 and 119 | 77 and 130 | 82 and 122 | 71 and 144 | 78 and 129 |

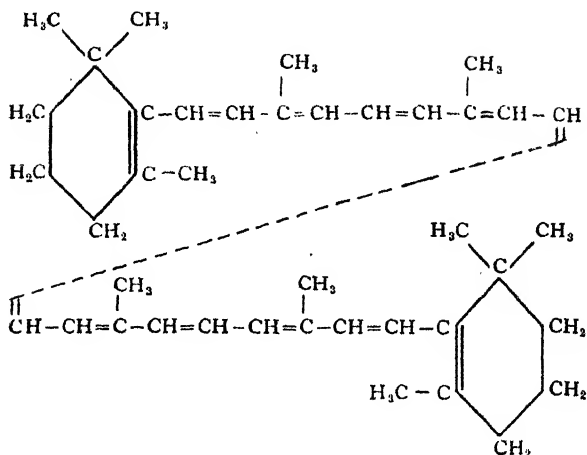
In the General Notices of the *B.P. Add. I* it is stated that "statements of the errors of these assays are based on the convention that, for practical purposes, a probability of 0.99 is equivalent to certainty. Thus, the statement 'limits of error (P = 0.99) 95 and 105%' means that it has been estimated that in 99 assays out of 100 the result will be greater than 95% and less than 105% of the true result."

For references to the therapeutic uses of the vitamins, see Vol. I, and for details of methods of analysis in urine and blood, see this volume, pp. 669 and 699.

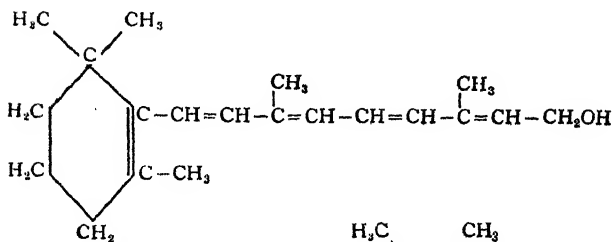
VITAMIN A

Chemistry of Vitamin A. It has been shown that, weight for weight, β -carotene has one half of the biological activity of the purest forms of vitamin A prepared (see crystalline vitamin A, p. 736). As its molecular weight is about double the molecular weight of vitamin A, it is probable that each molecule of β -carotene contains 1 active group and that it splits into 2 molecules, one of which is vitamin A and the other an inactive substance.

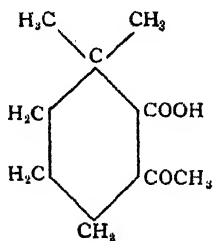
The formula accepted for β -carotene is



The formula accepted for vitamin A is



If geronic acid is written thus:—



it suggests how the decomposition of both β -carotene and vitamin A takes place when geronic acid is formed.—Karrer, Morf and Schöpp, *Helv. chim. Acta*, 1931, 1431.

The characteristics of the three carotenes are summarised in the following table:—

| | M.p. | Max. Absorption in CS ₂ | $[\alpha]_{844}$ |
|--------------------|------|------------------------------------|------------------|
| α -carotene | 183° | 509, 477, 448 | + 323° |
| β -carotene | 183° | 520, 485, 450 | Inactive |
| γ -carotene | 174° | 533, 496, 463. | Inactive |

Extracts of carotene from natural products contain much more β -carotene than α -carotene. They contain very little, if any, γ -carotene.

α - and β -carotenes can be separated by filtering a light petroleum solution through a closely packed column of air-dried calcium hydroxide. A wide band of yellow β -carotene is retained in the column, and below it, separated by a white band of the calcium hydroxide, a narrower yellow band of α -carotene is formed. These parts of the column can be taken out separately and the carotenes removed by suitable solvents.

The blue colour produced by the action of antimony trichloride on α -carotene gives only one absorption band, i.e., 542 m μ . That produced by β -carotene also gives one only, at 590 m μ . Highly concentrated vitamin A may also be separated into two fractions by absorption in a column of calcium hydroxide:—

(1) The α portion (a few per cent. only) which shows maximum absorption at 270 m μ .

(2) The β portion (the main portion) which shows maximum absorption at 328 m μ .

The blue colour given by antimony trichloride with the α portion shows at first only one band at 580 m μ but soon shows a band at 620 m μ . The β -portion shows at once a band at 620 m μ .—Karrer, Walker, Schöpp and Morf, *Nature, Lond.*, ii/1933, 26.

Liquor Vitamini A Concentratus (B.P. Add. II). Determined by the assay for vitamin A in relation to the standard preparation for vitamin A, it contains in 1 g. 50,000 units of vitamin A activity, and contains not more than 500 units per g. of antirachitic activity (vitamin D). Acid value, not greater than 2.5.

Oleum Vitaminatum (B.P. Add. II). Contains in 1 g. 1000 units of vitamin A activity and 100 units of antirachitic activity (vitamin D). Acid value, not greater than 2.5. Unsaponifiable matter, not more than 2%.

Oleovitamina A et D (U.S.P. XII) contains in 1 g., natural vitamin A equivalent to 850 to 1100 U.S.P. units of vitamin A and natural or synthetic vitamin D equivalent to 85 to 110 U.S.P. units of vitamin D.

Oleovitamin A (U.S.P. XII) contains vitamin A (obtained from natural animal sources) 50,000 to 65,000 U.S.P. units in each g., and not more than 1000 U.S.P. units of vitamin D.

Liquor Vitaminorum A et D Concentratus (B.P. Add. II). Contains in 1 g., 50,000 units of vitamin A activity, and 5000 units of antirachitic activity (vitamin D). Acid value, not greater than 2.5. Determined by the assay of vitamin A in relation to the standard preparation of vitamin A and by the biological assay of antirachitic vitamin (vitamin D) in relation to the standard preparation of vitamin D.

Oleovitamin A et D Concentrata (U.S.P. XII) contains in each g., 50,000 to 65,000 U.S.P. units of vitamin A and 10,000 to 13,000 U.S.P. units of vitamin D. Assayed by the U.S.P. XII process for vitamins A and D.

Stability of Vitamin A. Vitamin A survives the high temperatures of distillation of concentrates, 137° under 0.00001 mm. pressure and it is not destroyed by the ordinary cooking processes. The purest concentrates are also highly resistant to aerial oxidation at high temperatures. Both canned and frozen foods retain their vitamin A content for long periods, but rancid fats have a catalytic effect on their decomposition.

The weight of a vitamin A concentrate heated in air or oxygen increases through absorption of oxygen. The alcohol is probably oxidised to aldehyde and the unsaturated linkages to peroxides which are immediately destroyed and form aldehydes and ketones which polymerise. Some oxidation products were devoid of biological activity but still showed spectroscopic absorption at 328 mμ and gave purple colours in the SbCl₅ test, which would probably account for certain discrepancies between biological and spectrophotometric values of some concentrates.—F. A. Robinson, *Biochem. J.*, 1938, 32, 807.

The vitamin content of canned foods is not significantly inferior to that of fresh foods. As in the course of the canning process the air in the cans is reduced to vanishing point the thermolabile vitamins, which can withstand heat in the absence of oxygen, are little affected by the heating process.—*Brit. med. J.*, i/1934, 629.

While the vitamin in butter is not diminished by exposure to 120° for 4 hours, it is in the same period greatly diminished and in 12 hours completely destroyed if the butter is thoroughly aerated during the heating, i.e., one must conclude that, though fairly resistant to heat, this vitamin is readily destroyed by oxidation.—*Brit. med. J.*, i/1921, 237; i/1922, 236. Heating at 120° and 32 hours aeration destroys it.—E. Mellanby, *Brit. med. J.*, i/1924, 895.

Vitamin content of Australian, New Zealand and English butters.—M. E. F. Crawford, E. O. V. Perry and S. S. Zilva, *Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 175, 1932.

The vitamin A potencies of various vegetables have been estimated quantitatively in terms of the international unit by comparison with one sample of cod-liver oil of known potency, a test on the oil being made simultaneously with the test on each food substance. The boiling of the vegetables in a manner similar to that used in ordinary cooking did not destroy any vitamin A. Boiled carrots, cabbage and runner beans have been shown to be valuable sources of vitamin A, in view of the amount that can readily be eaten. Their potencies are about one-third, one-seventh, and one-tenth respectively of an average sample of summer butter, which contains 60 units per g.—K. H. Coward and B. G. E. Morgan, *Brit. med. J.*, ii/1935, 1041.

Effect of Solvents on Vitamin A Potency

Solutions containing 0.2 mg. of carotene per millilitre, with and without 0.02 g. of hydroquinone per millilitre, were stored in tightly stoppered test-tubes at 4°. The best results were obtained with cottonseed oil as solvent, with which there was a loss of 10% in 5 months, while olive, corn and coconut oils caused 25

to 50% destruction, not lessened by hydroquinone. With cottonseed oil at 4°, but exposed for 5 minutes daily to air and light to simulate the exposure during feeding tests, the loss during 5 months was 48%. Organic solvents were unsatisfactory. Destruction of the carotene can occur either by oxidation or by conversion to achroocrotene.—C. A. Baumann and H. Steenbock, *J. biol. Chem.*, 1933, 101, 561.

A solution of 0.2% of carotene in olive oil containing 0.1% of hydroquinone loses approximately half its vitamin A potency in a year; the effects of light and temperature are described.—R. G. Turner, *J. biol. Chem.*, 1934, 105, 443.

The choice of solvent is of importance in connection with the vitamin A activity of carotene and cod-liver oil.—F. J. Dyer, K. M. Key and K. H. Coward, *Biochem. J.*, 1934, 28, 875.

Vitamin A given as the free alcohol was absorbed from the gut of a human patient more completely than carotene.—J. C. Drummond, M. E. Bell and E. T. Palmer, *Brit. med. J.*, 1/1935, 1208.

Vitamin A dissolved in liquid paraffin is not completely absorbed, and if the paraffin comes into intimate contact with the carotene of the food, that is dissolved and excreted with the paraffin in the faeces.—O. Anderson, *Hospital-stidende*, 1938, 81, Supplement, 29-41.

Biological Determination of Vitamin A. The following is an abbreviated account of the method of assay given in the *B.P. Add. I*. Vitamin A is generally determined by its power to make rats resume growth after they have ceased to grow on a diet containing all factors known to be necessary for growth except vitamin A. A diet suitable for this purpose consists of:—

| | | | |
|-------------------------|-----|-----|-----|
| Sodium caseinate | ... | ... | 15% |
| Dextrinised rice starch | ... | ... | 73% |
| Dried brewers' yeast | ... | ... | 8% |
| Salt mixture | ... | ... | 4% |

In addition, about 10 units of vitamin D per week are given to each rat, for vitamin D is essential for growth as well as for calcification of bone.

The salt mixture consists of:—

| | | | |
|------------------------|-----|-----|--------------|
| Sodium chloride | ... | ... | 23.4 grammes |
| Magnesium sulphate | ... | ... | 24.6 " |
| Sodium phosphate | ... | ... | 35.8 " |
| Potassium phosphate | ... | ... | 69.6 " |
| Calcium acid phosphate | ... | ... | 68.8 " |
| Calcium lactate | ... | ... | 15.4 " |
| Iron citrate | ... | ... | 6.0 " |
| Potassium iodide | ... | ... | 0.2 gramme |

The diet may include also 15% of a vitamin-A-free fat in place of 15% of starch.

Young rats weighing about 30 g. are given the above diet until they cease to grow. Each rat is weighed twice weekly. When three successive half-weekly weighings have shown that its weight has not increased by more than 2 grammes it is allocated to one of four or five groups arranged so as to include equal numbers from each litter and equal numbers of males and females. Two of the groups are used for testing two doses of the standard preparation (e.g., 1 unit and 3 units), and the other groups for testing two or three doses of the cod-liver oil being tested (e.g., 0.5, 1 and 2 mg.). The doses may be given daily or twice a week in equivalent amount, suitable solutions being made so that the required dose can be

administered as one or more drops. The rats are weighed once a week for three weeks, and the average increases in weight in the rats of the different groups are then determined. Comparisons are drawn between the groups receiving the cod-liver oil being tested and those receiving the standard preparation, the activity of the cod-liver oil being calculated in terms of the standard preparation. The range of doses proposed for the standard preparation and the cod-liver oil will be suitable for samples of cod-liver oil whose potencies range from about 500 units per gramme (when the doses 2 mg. of cod-liver oil and 1 unit of the standard preparation give equal results) to about 6000 units per gramme (when the doses 0.5 mg. of cod-liver oil and 3 units of the standard preparation give equal results).

Only two groups of 10 rats each need be used if the relation between the average increase in weight and dose of vitamin A given has been previously determined. The rats in these groups then receive respectively 2 mg. of the cod-liver oil and 2 units of the standard preparation. If these groups give equal average increases in weight the potency of the oil is 1000 units per gramme; otherwise, the potencies of the doses are not directly proportional to the mean increases in weight but to the amounts of vitamin A, previously determined by the special experiment as corresponding to the two mean increases in weight.

Limits of Error. In an experiment in which 10 rats (5 males and 5 females) receive the standard preparation and 10 rats (5 males and 5 females) receive the preparation being tested, and in which the mean responses are equal, the limits of error ($P = 0.99$) are 30 and 339% for a three weeks' test and 37 and 272% for a five weeks' test.

It is essential to dilute the cod-liver oil with the same oil that is used for dissolving the standard. Coconut oil and arachis oil have been found suitable diluents.

An economy of animals and labour may be effected if many tests are to be carried out by first constructing a "*curve of response.*" Five groups of about 12 rats each (6 males and 6 females), prepared as described above, are given daily doses of 0.5, 1.0, 2.0, 4.0, and 8.0 mg. respectively of a particular sample of cod-liver oil, every rat in any one group being given the same dose. At the end of the test period (say, 3 weeks), the average gains in weight of the different groups are found and plotted against the dose of cod-liver oil given. The resulting curve will probably be logarithmic. It is then used as follows. In every fresh test, one group of 8 to 10 rats prepared in the usual way is given a daily dose of, say, 1 mg. of cod-liver oil; another group of rats prepared in the same way is given a daily dose of, say, 2 units of the standard. The mean increases in weight in 3 weeks of the different groups of rats are calculated. The abscissa of the curve corresponding to each mean increase in weight is found. The ratio of these two abscissæ gives the ratio of the vitamin A potency of the doses of cod-liver oil and standard respectively, from which may be calculated the vitamin A

potency of the oil. It should be stated as the number of International Units of vitamin A per gramme of cod-liver oil.

More often nowadays, the rats prepared for a determination of vitamin A are divided into four groups, two groups being given two doses (in the ratio 2 : 1) of the standard and the other two groups two doses (in the ratio 2 : 1) of the substance under examination. The slopes of the two curves of response so obtained (increase in weight against the \log_{10} of the dose given) are averaged and used as the slope for determining the relative potency of unknown to the standard. The horizontal distance between the parallel curves through the "mid points" of the curves found experimentally is the log of the ratio of the vitamin A contents of the doses of unknown and standard given.

The limits of error in the biological assay of vitamin A using the technique described by Morgan (*Biochem. J.*, 1934, 28, 1178) for P = 0.99 with 10 pairs of rats are 74 to 135%. The replacement of dextrinised rice starch in the B.P. Add. I diet by 30% of cocoanut-cake meal made it equal in all respects to Morgan's diet, which causes better growth during the depletion and test periods.—N. T. Gridgeman, H. Lees and H. Wilkinson, *J. Soc. chem. Ind.*, 1940, 120.

The curative method of assay for vitamin A involves a preliminary depletion period which causes variable pathological symptoms and, in consequence, serious discrepancies.—J. B. Orr and M. B. Richards, *Nature, Lond.*, 1/1934, 255.

Chemical Determination of Vitamin A. The reaction of arsenic chloride or antimony chloride with fish-liver oil and concentrates (transient blue colour) is probably due to vitamin A, but other substances present in the natural oil interfere with the development of the blue colour to a variable extent. The test, therefore, can only be regarded as a qualitative one, or quantitative to a very limited extent, viz., an oil giving a deep blue colour is probably rich in vitamin A, one giving a faint blue, or no blue at all, is probably nearly or quite devoid of vitamin A. But of two oils giving the same colour value, one may contain four times as much vitamin A as the other when measured biologically.

The blue value of a concentrate runs more nearly parallel with the biological value than the blue value of the oil itself. For crystalline vitamin A the Lovibond blue unit, determined by the antimony trichloride test, is equivalent to approximately 30 international units. The best method of preparing the unsaponifiable fraction of an oil is that recommended by the Permanent Commission on Biological Standardisation. (*See Evers and Smith, Quart. J. Pharm.*, 1934, 477.)

The value of the antimony trichloride test has been enhanced by the development of the photo-electric colorimeter, which enables the elusive point of maximum intensity of the blue colour to be measured by a galvanometer.—W. E. Eddy, *Chem. & Ind.*, 1939, 38.

Spectroscopic Estimation of Vitamin A. Vitamin A in fish-liver oils and in concentrates shows selective absorption at 328 m μ . The intensity of absorption at this wave-length of concentrates and of oils of more than 10,000 International Units of vitamin A per gramme may be taken as a quantitative measure of vitamin A. Cod-liver oils contain substances which interfere with the measurement of absorption and, therefore, the vitamin A in cod-liver oils

should be measured in the unsaponifiable fraction of the oil, not on the oil itself. The measurement is made by a spectrophotometer. It is always made on a 1% solution in alcohol or in cyclohexane (chloroform is unsuitable) contained in a quartz cell through 1 cm. depth of solution. The result is expressed as the value $E_{1\text{ cm.}}^{1\%}$ ("extinction coefficient"). It is the value of $\log I_0/I$ where I_0 is the incident light and I is the emergent light, e.g., a 1% solution of a concentrate in alcohol transmitted 5% of the incident light and absorbed 95%. Its value is, therefore, $\log I_0/I = \log \frac{100}{5} = 1.301$. An oil that transmitted 10% and absorbed 90% of the incident light would have the value, $E_{1\text{ cm.}}^{1\%} = \log \frac{100}{10} = 1.0$.

The measurement of absorption at $328\text{ m}\mu$ expressed as $E_{1\text{ cm.}}^{1\%}$, and made under certain defined conditions, may be a reliable method for measuring the vitamin A content of liver oils and concentrates. As a means of converting values obtained for $E_{1\text{ cm.}}^{1\%}$ $328\text{ m}\mu$ into a figure representing the International Units of vitamin A per gramme of the material examined, the factor 1600 is recommended for adoption. It is desirable that, when a figure expressing the biological potency of a preparation has been derived by the use of this calculation, the fact should be stated. —Reprot of the Second International Conference on Vitamin Standardisation, *Bull. Hlth Org., L.o.N.*, 1934.

A carefully planned experiment carried out by workers in nine different laboratories in Great Britain and the United States with a halibut-liver oil and a concentrate prepared from it showed that the biological value of the oil as stated in international units of vitamin A was 1470 times the $E_{1\text{ cm.}}^{1\%}$ value. (A later calculation by improved methods changed this factor to 1570.)

The result, therefore, provided no support for changing the value of 1600 recommended by the International Conference (1934) for the conversion factor. Determinations of the $E_{1\text{ cm.}}^{1\%}$ value of the remainders from the solutions used in the feeding tests showed that the oil itself had lost little or no activity during the test but that the concentrates had lost enough activity to account for the lower biological values found. It is suggested that other concentrates may have behaved in the same way, which would account for the low biological values and, therefore, for the low conversion factors for concentrates found by some workers.—E. M. Hume, *Nature, Lond.*, i/1937, 467.

A second collaborative experiment yielded no good evidence for departing from the figure of 1600 for the "conversion factor" adopted by the International Conference in 1934.—E. M. Hume, *Nature, Lond.*, i/1939, 22.

A third collaborative experiment on the potency of vitamin A β -naphthoate gave a value of 1770 for the conversion factor. American workers, however, repeatedly find a conversion factor round about 2000, but their biological values are determined by comparison with the first U.S.P. Reference Oil, whose potency, 3000 international units per gramme, was determined by comparison with the first International Standard for vitamin A (1931). This proved to be a mixture of carotenes and the second International Standard of Reference, pure β -carotene, was perhaps imperfectly related to the first standard. The value obtained for the vitamin A potency of the U.S.P. Reference Oil in the second collaborative experiment of this series of tests against the second International Standard was 2600 with a conversion factor of 1820. The ratio of this figure, 2600, to the reputed value 3000 is the same as the ratio of the average conversion factor found in the three collaborative experiments (i.e., 1740)

to the conversion factor advocated by the U.S.P. The discrepancy is of greater importance in commercial transactions than in scientific work or the clinical use of oils.—E. M. Hume, *Nature, Lond.*, i/1943, 536.

There is a substance in whale-liver oil which possesses biological activity, but of a lower order than vitamin A itself. Maximum absorption is at 285 to 290 $m\mu$. The presence of this substance in a liver oil increases the absorption at 328 $m\mu$ and would therefore falsify a determination of vitamin A based on its absorption at 328 $m\mu$. The absorption of any oil at 290 $m\mu$ should therefore be determined and expressed as a percentage of the absorption at 328 $m\mu$. If this is more than 75%, the oil should be regarded with suspicion.—R. T. M. Haines and J. C. Drummond, *Analyst*, 1938, 335.

Solutions may be prepared in ethyl or isopropyl alcohol, but the determination should be performed within an hour of making. If a condensed spark is used as a light source, it needs to be kept very accurately aligned; a hydrogen tube is preferable from this point of view. The photographic emulsion is best processed to a density of 0.6 for visual comparison. Unsaturated fatty acids can interfere, particularly with low potency oils, and also metallic substances such as copper and iron oleates. Numerous determinations on seven oils showed that the mean of two spectrophotometric results should be within 2 per cent. of the true value. The mean conversion factor from $E_{1\%}^{1\text{cm}}$ to U.S.P. units was found to be 2137.—D. T. Ewing *et al.*, *Industr. Engng Chem. anal. Edn.*, 1940, 639.

Measurements of the absorption curves showed that on storage for some months the absorption of both oil and unsaponifiable matter decreased. The shapes of the absorption curves were the same for the unsaponifiable matter of the reference oil, distilled vitamin A esters and their unsaponifiable fraction, but a commercial concentrate showed a slight double peak at 280 $m\mu$ like some cod-liver oils.—R. L. Macfarlan *et al.*, *Industr. Engng Chem. (anal. Edn.)* 1940, 645.

Free vitamin A (alcohol) in polar solvents seems to form some complex yielding extinction coefficients greater than those obtained in non-polar solvents, whereas the vitamin A ester forms no such complex. It is therefore recommended that a non-polar solvent should be used in estimations of the vitamin A in the non-saponifiable fraction of an oil.—D. C. M. Adamson and N. Evers, *Analyst*, 1941, 106.

With the aid of a Beckman quartz photo-electric spectroscope, the influence of solvent on the ultra-violet absorption maximum of vitamin A has been examined. Special reference is made to differences in behaviour between ester and alcohol forms of vitamin A.—K. Morgareidge, *Industr. Engng Chem. (anal. Edn.)*, 1942, 700.

Vitamin A₂. Differences in the chromogenic properties of fresh-water and marine fish-liver oils. The 693 $m\mu$ band developed on the addition of antimony trichloride to the liver oil of a fresh-water fish may indicate the existence of a second vitamin A with six conjugated ethenoid linkages and strong absorption at 345 to 350 and 280 to 285 $m\mu$. Comparison of biological and spectrographic values is being made, but until both biological and spectrographic data have been accumulated and examined, the accurate determination of the vitamin A content of liver oils of fresh-water fish, by physico-chemical methods, is not possible.—A. E. Gillam, I. M. Heilbron, E. Lederer and V. Rosanova, *Nature, Lond.*, ii/1937, 233.

A possible vitamin A₂. The chromogenic substance giving rise to the substance with absorption at 693 $m\mu$ on treatment with SbCl₃ has been found in extracts from gold-fish eyes and brown trout. Direct absorption spectra showed three broad bands with maxima 470, 350 and 287 $m\mu$, these varying in intensity with the 693 $m\mu$ band in the colour test.—J. R. Edisbury, R. A. Morton and G. W. Simpkins, *Nature, Lond.*, ii/1937, 234. See also *Biochem. J.*, 1938, 32, 118; E. L. Gray, *J. biol. Chem.*, 1939, 131, 317; J. A. Lovern *et al.*, *Biochem. J.* 1939, 33, 325; *ibid.*, 330.

Vitamin A₂ probably functions in fresh-water fish in the same way as vitamin A functions in sea-fish. It has been found only in those mammals or birds which have been known to feed on fresh-water fish. Growth tests on rats indicate that vitamin A₂ has some biological activity for the rat, but there is no suggestion at present that it is necessary for the human being.—*Lancet*, ii/1939, 345.

For vitamin A content of fish oils, see pages 288 and 769.

Crystalline Vitamin A. Vitamin A alcohol has been obtained by repeated fractional molecular distillation of concentrates of vitamin A, substantially free from sterols, in a cyclic still of the type described by Hickman. It crystallises from methyl alcohol at low temperatures in pale yellow crystals. When it is allowed to warm up slowly in an evacuated tube it melts at about 8° to a liquid which evolves vapour which may prove to be solvent of crystallisation and simultaneously becomes more viscous. When completely free from solvent in this manner, the product had a Carr-Price value of 92,000 and $E_{1\text{cm}}^{1\%}$ 328 $m\mu$ = 1820 (in ethyl alcohol). The stearate, 4-nitrobenzoate, diphenylacetate and acetate have been prepared from highly purified vitamin A, but have not been obtained crystalline. The crystalline anthraquinone-2-carboxylate and 2-naphthoate of vitamin A have been prepared from material purified by molecular distillation. These esters were found to resemble those prepared by Hamano from undistilled concentrates. Biological tests of these esters, carried out in two different laboratories, gave a value of 3,181,000 i.u. per gramme of vitamin A in the first ester and 3,424,000 i.u. per gramme of vitamin A in the second ester. Since β -carotene contains, by definition, 1,670,000 i.u. of vitamin A activity per gramme, vitamin A itself is twice as active as β -carotene, weight for weight.—S. W. F. Underhill and K. H. Coward, *Biochem. J.*, 1939, 33, 589.

Crystalline vitamin A has been isolated from shark liver, ling cod liver, California jewfish liver and halibut viscera oils as yellow prisms melting at 63° – 64° . Evidence was obtained indicating that the crystalline vitamin A of Holmes and Corbet (m.p. 7.5° – 8°) contained methyl alcohol. Crystalline vitamin A had a provisional biological potency of 4,300,000 U.S.P. XI units per g. Its conversion factor, 2460, was higher than the conversion factor of vitamin A in fish-liver oils. The extinction coefficient of crystalline vitamin A at 328 $m\mu$ was 1780. The extinction coefficient of the vitamin A antimony trichloride blue colour at 622 $m\mu$ was 4800.—J. D. Baxter and C. D. Robeson, *J. Amer. chem. Soc.*, 1942, 2416.

The extinction coefficients of crystalline vitamin A acetate, vitamin A palmitate and di-vitamin A succinate, prepared by esterification of crystalline vitamin A, have been determined at 328 $m\mu$. Similar determinations at 620 $m\mu$ of the antimony trichloride blue colours have also been made. The biological potency of the esters, adjusted for differences in molecular weight, was the same as that of crystalline vitamin A. **Vitamin A acetate** was the most resistant of the crystalline preparations to atmospheric oxidation and appears to be the most stable crystalline ester of vitamin A yet prepared.—J. D. Baxter and C. D. Robeson, *J. Amer. chem. Soc.*, 1942, 2407.

Attention is directed by the authors to the inconsistency of using a physical property that carotene does not possess, i.e., ultra-violet absorption, for calculating activity in international units which are defined in terms of carotene when evidence is available that vitamin A and β -carotene are not of equal

For determination of thiamine hydrochloride in various preparations the biological assay of the *U.S.P. XII* and the reference standard thiamine hydrochloride may be used. The activity of 3 micrograms of the *U.S.P.* reference standard thiamine hydrochloride is one *U.S.P.* unit of vitamin B₁, which is equal in value to the international unit of vitamin B₁.

Pulvis Vitamini B₁ (B.P. Add. I). Determined in relation to the standard preparation of antineuritic vitamin (vitamin B₁) by the biological assay of antineuritic vitamin (vitamin B₁) contains 100 units of antineuritic vitamin (vitamin B₁) per gramme.

Tabellæ Thiaminæ Hydrochloridi (U.S.P. XII). Contain 95 to 120% of the labelled amount of C₁₂H₁₇ClN₄OS.HCl, including all tolerances. In the assay, a weighed quantity of powdered tablets is dissolved in approximately N/100 hydrochloric acid and an aliquot of the solution treated with water and 0.78% w/v alcoholic solution of phenol. Thymol blue indicator and N/1 sodium hydroxide are added until alkaline. After the addition of a freshly prepared solution of diazotised *p*-aminoacetophenone, the solution is allowed to stand in the dark for 2 hours, and is then shaken vigorously with an accurately measured volume of xylene. The colour of the xylene solution is viewed transversely against a white background with control tests prepared concurrently using thiamine hydrochloride reference standard and containing 95, 105 and 120% of the labelled amount of thiamine hydrochloride in the quantity of tablets used. The colour of the xylene solution is not lighter than the control containing 95% and not darker than the control containing 120% of thiamine hydrochloride reference standard.

Perpolitiones Oryzæ (U.S.P. XII). Rice polishings are required to contain not more than 40% of starch and not more than 10% of rice hull or other foreign matter. Starch is determined by hydrolysing the water-insoluble fraction with hydrochloric acid, neutralising, reduction of alkaline cupric tartrate solution and weighing the dried residue of cuprous oxide. Foreign matter is determined by passing through a No. 30 sieve. 1 ml. of Extractum Oryzæ, *U.S.P. XII*, contains not less than 20 *U.S.P.* units of vitamin B₁, and represents approximately 14.5 g. of rice polishings.

Stability of Vitamin B₁. In faintly acid or in acid media, the resistance of vitamin B₁ to heat is considerable. In baking bread or biscuits, when the temperature of the interior of the loaf does not rise above 100°, no serious diminution in vitamin B₁ content may be expected, provided that yeast has been used as the raiser, not alkaline baking powders. Crude concentrates of vitamin B₁ have been kept in 99% alcohol at pH 1.0 to 2.0 at room temperature for 3 years, but some highly purified preparations gradually lost their activity under these conditions.

Alkalis, on the other hand, cause ready destruction of vitamin B₁ even at low temperatures. It is not oxidised by exposure to air or ozone. In acid solution it is stable to hydrogen peroxide, and to potassium permanganate.

Biological Determination of Vitamin B₁. Vitamin B₁ is determined either on pigeons or on rats. The cure of retracted neck in pigeons must be considered a more specific test for vitamin B₁ than the resumption of growth in rats which have ceased to grow on a deficiency of that factor.

The determination of vitamin B₁ by the use of pigeons is carried out as follows:—

About a hundred pigeons are given a diet of polished rice in cages provided with open wire mesh screens to prevent

the birds from having access to their faeces. The faeces often contain vitamin B₁ even when the diet does not and pigeons and rats will eat their own faeces when otherwise deprived of that factor. Fresh water is given daily. In 3 to 4 weeks many pigeons fed on a diet of polished rice will develop retracted neck, quite suddenly and generally overnight. Two or three doses of the substance under examination should be tested against one or two doses of the standard. Generally a dose of 30 mg. of the standard will produce a cure in 50% of the birds used for this test. In any test in which the result is necessarily either positive or negative (e.g., cure of retracted neck or failure to cure retracted neck; death of the animal or recovery of the animal) it is desirable to aim at a comparison of doses which bring about a 50% positive result. Thus it should be the aim in a test for vitamin B₁ in pigeons to discover the dose of test substance and of standard which, in the particular lot of pigeons used, will both bring about 50% of cures in the groups of birds given those doses. Eight or ten birds should be used in each group. Often only about half of the pigeons given the diet of polished rice develop retracted neck; therefore it is necessary to start with about twice as many pigeons as it is proposed to use in the test.

Comparison of the duration of cure of the same pigeons may also be made, and will, within the limits of experimental error, give the same result as the comparison made by the percentage of birds cured.

Curves of response may be constructed for this test in the same way as the curve of response for the estimation of vitamin A, if many estimations of vitamin B₁ are to be made. When this has been done one dose of the standard only need be tested simultaneously with the test of one dose of the substance under examination. The results are then calculated as in the vitamin A test.

The determination of vitamin B₁ by the growth response of rats. The following is an abbreviated account of the method given in the *B.P. Add. I*.

About ten young rats of 50 g. weight are given a diet containing all factors known to be necessary for growth except B₁. A suitable diet for this purpose consists of the following mixture thoroughly cooked by steaming for about three hours:—

| | | | | |
|-------------------------------|-----|-----|-----|-------------|
| Sodium caseinate | ... | ... | ... | 100 grammes |
| Rice starch | ... | ... | ... | 300 " |
| Salt mixture | ... | ... | ... | 25 " |
| Arachis oil or Cottonseed oil | ... | ... | ... | 75 " |
| Water | ... | ... | ... | 500 " |

In addition about 5 drops of a good sample of cod-liver oil are given to each rat daily directly into its mouth to supply vitamins A and D. Vitamin B₂ may be provided by administration of 1 ml. of an autoclaved extract of yeast. Throughout the whole test the rats' cages are provided with wire mesh grids to prevent the rats from having access to their faeces.

Rats fed on this diet will cease to grow in 2 to 3 weeks. As the rats become steady in weight, they are divided into two groups, each rat being put in a separate cage. The rats of each group receive respectively 10 mg. daily of the substance being tested or 10 mg. (1 unit) of the standard preparation, for a period of four weeks. If the average increase in weight is then the same for both groups the vitamin B_1 of the substance being tested is equal to that of the standard preparation; otherwise the test is repeated using larger or smaller doses. Alternatively, for the first trial two doses of the substance being tested may be given and fourteen rats used. In each trial two rats should receive no dose; there should be a gradual decline in weight ending usually in convulsions.

The activity of the preparation being tested is calculated from the dose which gives a result equal to that given by 1 unit of the standard preparation.

Limits of Error. Increase in weight method. In an experiment in which five rats receive the standard preparation and five rats receive the preparations being tested, and in which the mean responses are equal, the limits of error ($P = 0.99$) are 65 and 154% for a 3 weeks' test.

Thiochrome Test for Determination of Vitamin B_1 in Foodstuffs. The process is based on the method of Jansen in which the vitamin is converted into the strongly fluorescent compound thiochrome by alkaline oxidation, and the intensity of the fluorescence is then measured by means of a photo-electric cell in a Cohen fluorimeter.

The finely ground or minced material is placed in 1% HCl and the mixture vigorously stirred, just brought to the boil, then allowed to cool and made up to a convenient volume. Four 3 ml. aliquots of the filtered extract are added to a mixture of 2 ml. of methyl alcohol, 1 ml. of 30% sodium hydroxide and 0, 0.8, 1.0, and 1.2 ml. of 1% potassium ferricyanide. The solutions are well mixed and each is shaken with 13 ml. of isobutyl alcohol and centrifuged. 10 ml. of the isobutyl alcohol layers are used for measurement of the fluorescence. The reading obtained with the solution containing no ferricyanide is subtracted from the other readings obtained in order to correct for any non-specific fluorescence. If the results on the three solutions containing ferricyanide do not agree closely, the tests must be repeated, using other proportions of ferricyanide until maximum fluorescence is obtained. The galvanometer is standardised against the international standard adsorbate. For the determination of vitamin B_1 in urine, concentration must first be effected by adsorption on fuller's earth since the amount present is too small for direct determination. 100 ml. of sample is brought to pH 4.5 with hydrochloric acid and treated with 1 g. of fuller's earth. 20 mg. aliquots of the dried adsorbate are used for the assay.—M. A. Pyke, *Biochem. J.*, 1937, 1958. Also: *Nature, Lond.*, 1/1938, 1141.

Modifications and improvements are suggested. The method, originated by Jansen (*Rec. trav. chim.*, 1936, 55, 1046) and improved by various workers, gives results in close agreement with biological assays, and has been applied to various types of natural products, including grains and vegetables.—R. T. Conner and G. J. Straub, *Industr. Engng Chem. (anal. Edn.)*, 1941, 380.

Sample extracted with 2% acetic acid and in a boiling water-bath for 15 minutes. Subsequent treatment with Taka-Diastase unnecessary with flour.—Shetlar and Lyman, *Cereal Chemistry*, 1941, 666.

Finely ground sample can be extracted with boiling 0.4N sulphuric acid (pH 1 to 2) for 1 hour with continuous stirring. Cocarboxylase hydrolysed by treatment with Taka-Diastase or clarase mixture at 45° for 2 hours in a buffer solution of pH 4.5. An aliquot portion of the cooled and centrifuged mixture is pipetted into 2% acetic acid, heated to boiling and adsorbed on column of activated 60 to 80 mesh Decalco (synthetic zeolite). The thiamine can then be eluted with hot 25% potassium chloride and oxidised under controlled con-

ditions as to quantities of potassium ferricyanide, sodium hydroxide and iso-butyl alcohol.—Conner and Straub, *Industr. Engng Chem. (anal. Edn.)*, 1941, 31, 380.

Pyke's method found unsatisfactory with yeast. It is recommended that a 0.04N acetic acid solution of yeast be raised to boiling, cooled for 2 minutes, again brought to boiling and cooled to 37°. The pH is then adjusted to 4.0 to 4.5 and treated with 0.05 g. Taka-Diastase at 37° for 4 hours, diluted and centrifuged. A hot acid extraction (without hydrolysis with Taka-Diastase) is satisfactory if no part of the B_1 is present in phosphorylated form.—Dawson and Martin, *J. Soc. chem. Ind., Lond.*, 1941, 241. Dawson and Martin also prefer to use the visual method for estimation of thiochrome in meals and breads. They find that with some materials (e.g., brown meals) it is advisable to adjust the pH after 2 hours incubation with the pepsin-hydrochloric acid solution and carry out the Taka-Diastase treatment after adjusting the pH with 2M sodium acetate-acetic acid buffer (pH 4.6).—*J. Soc. chem. Ind., Lond.*, 1942, 13.

Sample should be ground to at least 50 mesh and pepsin solution adjusted to pH of 2.0 to 2.5. Taka-Diastase digestion carried out at pH 4.7. Interfering substances removed by refluxing with methyl alcohol, followed by treatment with 1 ml. of 30% hydrogen peroxide after addition of potassium ferricyanide and sodium hydroxide. The need for control on the quantity of potassium ferricyanide is shown.—Johannson and Rich, *Cereal Chemistry*, 1941, 473.

Details for the oxidation of the extracted aneurine and the measurement of the intensity of fluorescence either visually or photo-electrically. Sodium hydroxide is added before the potassium ferricyanide, otherwise low results are obtained. Extraction of aneurine from flour can be carried out without enzyme digestion, simply with dilute hydrochloric acid.—Nicholls *et al.*, *Analyst*, 1942, 15 and 162.

The method of iso-butanol extraction of the aneurine oxidised to thiochrome depends on a constant ratio between the aqueous and iso-butanol components of the standard and test solutions—the extraction of thiochrome by iso-butanol from an aqueous phase is incomplete.—Booth, *J. Soc. chem. Ind., Lond.*, 1940, 181.

Colorimetric Determination in Pharmaceutical Preparations.

Thiamine will couple with diazotised *p*-aminoacetophenone to produce a purplish-red substance insoluble in water but soluble in organic solvents. The reaction has been adapted for the estimation of thiamine in pharmaceutical preparations colorimetrically. The standard solution consists of thiamine chloride 50 mg. dissolved in 500 ml. of 50% alcohol containing 1 drop of 4N HCl. Colourless test solutions are assayed by diluting until 1 ml. contains about 100 microgrammes of thiamine, and placing 1 ml. of the diluted solution in a test-tube calibrated at 10 ml. To this is added 2 ml. of 50% alcohol and the tube is placed in a water-bath at 60°. After one minute is added one ml. of diazo solution A (prepared by mixing 10 ml. of *p*-aminoacetophenone with 2 ml. of 0.1% sodium nitrite solution, cooling in ice and finally adding just prior to use 3 ml. of N NaOH). The solutions are mixed, allowed to stand at 60° for 2 to 3 minutes, cooled, one drop of 4N HCl added and the volume adjusted to 10 ml. with isopropyl alcohol. The colour is then compared with that of a reference solution prepared by mixing 1 ml. of distilled water, 1 ml. of standard thiamine solution, 1 ml. of 50% alcohol and 1 ml. of diazo solution A. Details are also given for applying the method to elixirs and tablets, and an addendum to the paper describes the modifications rendered necessary by the presence of ascorbic acid, since the latter substance prevents the development of the colour.—M. E. Auerbach, *J. Amer. pharm. Ass. (Sci. Edn.)*, 1940, 313.

Fermentation Test for Estimation of Vitamin B_1 and Aneurine.

Depends upon the stimulation in the rate of alcoholic fermentation by yeast. Both natural B_1 and synthetic aneurine are powerful accelerators. Yeast is added to the reacting solutions in sufficient quantity to ferment out the sugars as rapidly as they are formed and the carbon dioxide evolved is collected and measured at frequent intervals. The reaction mixture consists of 15 ml. of solution A (ammonium phosphate buffer— $\text{NH}_4\text{H}_2\text{PO}_4$ and $(\text{NH}_4)_2\text{HPO}_4$ in ratio 5:3—not more than 5% in reaction mixture), 15 ml. of solution B (dextrose solution of not more than 40% concentration); with salts of potassium, calcium, magnesium and traces of manganese and iron. Reaction mixture is placed in bottle, followed by 25 ml. yeast suspension and 45 ml. water. Initial

reading in gasometer made after 2 to 3 minutes—subsequent readings are made at convenient intervals, usually 15-minute periods. The solution or suspension under test (which should be sterilised and have an acid reaction prior to sterilisation) replaces a portion of the distilled water and the quantity should be equivalent to 2 to 5 gamma of the pure vitamin.

Fermentation stimulation is not specific for thiamine and caution is needed in interpreting the results; correlation with animal growth or with crystalline compound taken as standard, should be carried out periodically. 2-Methyl-5-ethoxymethyl-6-aminopyrimidine is an interfering stimulator.—Schultz, Atkin and Fry, *J. Amer. chem. Soc.*, 1937, 59, 2457.

The effect of stimulation by nicotinic acid is eliminated by including in all tests 1.0 mg. nicotinic acid; no further stimulation is obtained with larger amounts.—*J. Amer. chem. Soc.*, 1938, 60, 1514.

A further modification in which any stimulation not due to thiamine is eliminated by destruction of the thiamine with sulphite and the fermentation procedure repeated—any stimulation found in these conditions is subtracted from the original values with the untreated samples.—*J. Amer. chem. Soc.*, 1941, 63, 632.

A modified micro-fermentation method for the estimation of thiamine.—E. S. Josephson and R. S. Harris, *Industr. Engng Chem. (anal. Edn.)*, 1942, 755.

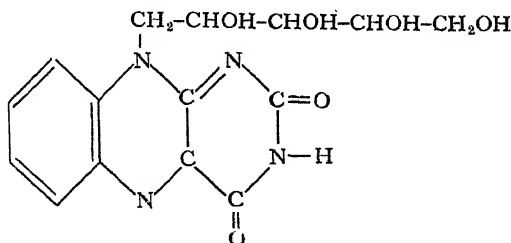
For determination of Vitamin B₁ in flour, see p. 818.

VITAMIN B₂

Chemistry of Vitamin B₂. The factor originally known as vitamin B₂ has for some time been recognised as consisting of at least three parts: (1) Riboflavin, for which the synonym vitamin B₂ may be retained; (2) Nicotinic acid, previously known as the P P factor; (3) Pyridoxine, previously known as vitamin B₆.

Riboflavin (*B.P. Add. VI*). Melts with decomposition at about $280^{\circ}\alpha_D$ at 20° , -110° to -130° , calculated with reference to the substance dried over sulphuric acid and determined within 30 minutes of the preparation of a 0.5% w/v solution in 1.5 ml. of N/10 alcoholic potassium hydroxide (carbonate free) and sufficient carbon dioxide-free water to produce 10 ml. Loss on drying over sulphuric acid, not more than 1.5%; ash, not more than 0.5%. Nitrogen content, by Kjeldahl method, 14.5 to 15.2%, calculated with reference to the dry substance. A suitable micro-analytical method may be used if desired. Riboflavinum, *U.S.P. XII*, complies with the same standards; α_D at 25° , determined on a 0.5% solution prepared as above, -112° to -122° .

Riboflavin has the formula:



Evidence given to show that a disease occurring in Malaya (particularly in institutions) in which the main lesions in the early stages are eczema of the scrotum and of the angles of the mouth, and in the late stages combined degeneration of the cord and poor vision, is due to avitaminosis B₂. It is curable in the early stages by the use of yeast or Marmite, fresh or autoclaved.—J. V. Landor and R. A. Pallister, *Trans. R. Soc. trop. Med. Hyg.*, ii/1935, 133.

The chief symptoms of ariboflavinosis are cheilosis, ocular lesions, seborrhoeic dermatitis and characteristic glossitis. Most of these symptoms clear up readily when treated with riboflavin; the ocular lesions, if not too advanced, also yield to the treatment. The daily dose of riboflavin used by different workers to bring about cure ranges from 2 to over 50 mg. This would indicate that the requirement for prevention of symptoms of ariboflavinosis is somewhat below 2 mg. a day and this would appear to be about the usual daily intake in the diet.—*Brit. med. J.*, i/1942, 156.

Riboflavin is essential to growth and to normal nutrition at all ages. When the food is poor in riboflavin for any considerable length of time digestive disturbances, nervous depression, general weakness and deterioration of tone, and poor condition of the eyes and skin are apt to develop; the incidence of infectious disease is likely to be increased, vitality diminished, life shortened, and the prime of life seriously curtailed by the unduly early development of the physical manifestations of old age. On the other hand, riboflavin has been found, like calcium, to confer increasingly beneficial results with increasingly liberal intakes up to levels of from two to four times as high as that of minimal adequacy for permanently normal nutrition. And it may prove of much significance for continuing human progress that further increases in the riboflavin content of the diet, beyond the zone in which the individuals of the first generation show immediately tangible response, still seem to confer additional benefit upon the offspring.—Sherman, *Chemistry of Food and Nutrition* (Macmillan, 1941).

Tabellæ Riboflavinæ (U.S.P. XII). Contain 95 to 120% of the labelled amount of $C_{17}H_{20}N_4O_6$, including all tolerances. Assayed by dissolving a weighed quantity of powdered tablets in water, allowing to stand in the dark, decanting or filtering in diffused light, and comparing in matched tubes the colour of the clear solution with control solutions prepared from reference standard riboflavin containing in the same volume 95, 105 and 120% of the quantity of riboflavin represented in the tablets taken for the assay. If the test solution is filtered, the control solutions must also be filtered. When viewed transversely against a white background the test solution is not lighter than the solution containing 95% and not darker than the solution containing 120% of riboflavin reference standard.

Stability of Riboflavin. Protected from light it is stable at ordinary temperatures. In solution riboflavin decomposes under the influence of both visible and ultra-violet light, the chain of reactions depending on pH, presence or absence of oxygen, intensity of illumination, temperature, etc. In the dark, acidified solutions of riboflavin are stable. For example, a solution of pH not above 6.5 may be kept at 100° in the dark for one hour without appreciable decomposition. Riboflavin is quite stable to oxidising agents, but is reduced by many reducing agents.—A. Osol, *Amer. J. Pharm.*, 1941, 268.

Determination of Riboflavin in Foodstuffs. A quantity of the food containing not more than 200 γ of flavin is weighed into a 250 ml. stoppered bottle and a mixture of 10 ml. of methanol (technical grade may be used) and 40 ml. of dilute hydrochloric acid is added. After shaking well the bottle is incubated at 37° for 48 hours with occasional shaking. The contents are then centrifuged—if large amounts of protein are present 1 ml. of concentrated hydrochloric acid is added first. The supernatant liquid is transferred to a 250 ml. distilling flask, the residue in the tube is repeatedly centrifuged with methanol until no more colour is extracted, and the mixed supernatant liquid and washings are distilled under reduced pressure until the alcohol has been removed. The heat should be derived from an electric heater covered with asbestos paper so as to prevent leakage of light, the progress of distillation being

observed by a red light. The occasional bubbling of air through the apparatus prevents reduction of flavin. When the volume is reduced to 40 ml., the liquid is transferred to a 250 ml. stoppered bottle, if necessary after centrifuging, and 3 g. of fuller's earth and 3 ml. of concentrated hydrochloric acid added. Shake well and place in a rocking machine for 1 hour. Again centrifuge, reject the supernatant liquid even if coloured, add 50 ml. of pyridine-methanol-water mixture (1:1:4) to the fuller's earth adsorbate in a 250 ml. stoppered bottle and shake in the rocking machine for 1 hour. Again centrifuge, transfer the clear liquid to a distilling flask, completely extract the fuller's earth by washing with successive quantities of methanol, adding the washings to the distilling flask, and distil until the volume is reduced to 2 ml. Transfer the residue to a small centrifuge tube, rinsing the flask thoroughly with acetone. Stir, again centrifuge, note the volume of supernatant liquid and transfer it to a test-tube. Adjust the pH to 7.0 by means of alkali (centrifuging again if necessary). The clear solution is made up to a definite volume and its fluorescent colour is compared against a riboflavin standard.—G. N. Murthy, *Indian J. med. Res.*, 1937, 1083.

Fluorometric method for determining the riboflavin content of foodstuffs.—A. Z. Hodson and L. C. Norris, *J. biol. Chem.*, 1939, 131, 621; also M. Swaminathan, *Indian J. med. Res.*, 1942, 30, 23.

The method of Hodson and Norris has been modified, and fair agreement obtained between results from this method and biological determination.—W. S. Jones and W. G. Christiansen, *J. Amer. pharm. Ass. (Sci. Edn.)*, 1940, 270.

After extracting and treating with clarex as for the thiamine determination, an aliquot containing 5 µg. of thiamine and 1 µg. of riboflavin is poured into 2 absorption tubes (one above the other). The upper tube contains activated 60 to 80 mesh Decalco to absorb the thiamine and the other contains activated Supersorb (fuller's earth) to absorb the riboflavin. This is eluted with 23 ml. of 20% pyridine in 2% acetic acid, followed by small portions of eluant to give 50 ml. To a 15 ml. aliquot in a brown bottle is added 1 ml. of 40% freshly prepared potassium permanganate and after 1 minute 3 ml. of hydrogen peroxide, shaken vigorously and stood until effervescence has ceased. Filter 511 for transmission of incident light and filter 351 for the fluorescent light are used for comparison with the standard solution of 0.0856 µg. of riboflavin per ml. in pyridine-acetic acid and give the highest reading with the lowest blank.—Corner and Straub, *Industr. Engng Chem. (anal. Edn.)*, 1941, 385.

Microbiological Method for the Assay of Riboflavin. Assay tubes are prepared from stock solutions: 50 ml. photolyzed sodium-hydroxide-treated peptone solution, 50 ml. cystine hydrochloride solution, 5 ml. yeast supplement, 5 ml. glucose, 2.5 ml. solution A (potassium hydrogen phosphate, potassium dihydrogen phosphate), 2.5 ml. solution B (magnesium sulphate heptahydrate, sodium chloride, ferrous sulphate heptahydrate, manganese sulphate heptahydrate), the pH is adjusted to 6.6 to 6.8 with sodium hydroxide and diluted to 250 ml. 5 ml. is placed in bacteriological test-tube, riboflavin extract added and made up to 10 ml. Assay tubes are autoclaved at 1 kg./sq. cm. for 15 minutes and cooled. For the inoculum, a stab from a stock culture of *Lactobacillus casei* is made into a sterile tube of basal medium to which has been added 0.5 to 1.0 µg. of riboflavin per 10 ml. Culture is incubated 24 hours at 37° and the cells centrifuged out aseptically and resuspended in an equal volume of sterile 0.9% sodium chloride solution. One drop of this suspension is used to inoculate each assay tube. Inoculated assay tubes are incubated at 37° to 40° for 1 to 3 days. Standard tubes are set up at the same time and standard curves constructed (0.0, 0.05 to 0.5 µg. riboflavin).

Response determined: (1) measurement of turbidity produced by growth of organism, using Evelyn photo-electric colorimeter, 540 mµ filter (unsuitable for turbid or highly coloured solutions), or (2) measurement of acid produced during growth—titration to pH 6.8 to 7.0 with N/10 sodium hydroxide, using bromothymol blue indicator.

Methods of extraction from natural products are given.—Snell and Strong, *Industr. Engng Chem. (anal. Edn.)*, 1939, 346.

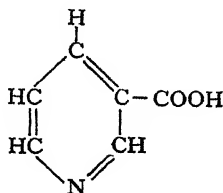
The presence of starch has a marked stimulatory effect on the growth of *Lactobacillus casei*—fictitious results produced in cereal products. Overcome by treatment of the aqueous extract with Taka-Diastase.—Andrews, Boyd and Terry, *Cereal Chemistry*, 1942, 55.

Determination in Liver Extracts. Pipette accurately 1 ml. of liquid extract into a 50 ml. separating funnel. In the case of extracts containing a

large amount of riboflavin, it is desirable to dilute the extract suitably with water and take 1 ml. of the mixture. Add 1 ml. of N/1 hydrochloric acid, mix, add 10 ml. of acetone slowly with continuous mixing. Add 10 ml. of chloroform, shake, allow to separate. Run off the lower layer into a 100 ml. separating funnel. Continue the acetone-chloroform treatment of the upper layer in exactly the same manner until the lower layer ceases to give a blue fluorescence when examined in "black light" (filtered ultra-violet radiation). Reserve the extracted upper layer. Extract the combined lower layers with 5 ml. portions of water until no more fluorescing material can be removed. Discard the extracted lower layer and combine the aqueous extracts, extracting with successive quantities of chloroform in order to remove any blue fluorescing material. Transfer the aqueous extract, freed from blue fluorescing material to a suitable round-bottomed flask, add the reserved upper layer and remove acetone by heating in a vacuum to a temperature of 35° to 40°. Add N/1 sodium hydroxide till just pink to phenolphthalein which is used as an internal indicator, and make up to a volume of 30 ml. Transfer to a shallow evaporating dish, adding an equal volume of N/1 sodium hydroxide. Expose to unfiltered ultra-violet radiation for a suitable time. Transfer the irradiated solution to a 200 ml. separating funnel, add a 20% solution of citric acid until the mixture is faintly acid to phenolphthalein, and extract with 10 ml. quantities of chloroform until no more fluorescing material is removed. Bulk the chloroform extracts to a suitable volume and compare the fluorescence of 10 ml. of this with prepared luminiflavin standard tubes.—G. E. Shaw, *Quart. J. Pharm.*, 1939, 541.

Acidum Nicotinicum (B.P. Add. IV). $C_5H_4N \cdot COOH = 123.1$. Loses not more than 1% at 100° and then contains not less than 99.5% of the pure acid. Determined by titration with N/10 sodium hydroxide to phenolphthalein. Arsenic limit, 2 parts per million. Lead limit, 10 parts per million. Residue on re-ignition with sulphuric acid, not more than 0.2%. The *U.S.P. XII* requires the same purity on the substance dried for 3 hours over sulphuric acid.

It has the formula:



Tabellæ Acidi Nicotini (U.S.P. XII). Contain 95 to 120% of labelled amount of $C_5H_4O_2N$, including all tolerances. Assayed by extracting four times with boiling neutral alcohol, dilution of the combined alcoholic extracts followed by evaporation and finally titrating against N/10 sodium hydroxide, using phenolphthalein indicator.

Determination of Nicotinic Acid in Foodstuffs. A hot aqueous extract of the test substance is deproteinised with lead acetate followed by sulphuric acid and decolorised at pH 10 with charcoal. The extract is brought to pH 7.5 and an aliquot portion treated with aqueous cyanogen bromide solution. After half an hour aqueous aniline solution is added and the greenish-yellow colour extracted with amyl alcohol. The colour is compared with a standard similarly prepared from nicotinic acid, and 0.01 mg. of nicotinic acid is easily detected. The method depends on the assumption that nicotinic acid and its pellagra-preventing derivatives constitute the major portion of the pyridine compounds present in foodstuffs.—M. Swaminathan, *Nature, Lond.*, 1/1938, 830.

König in 1904 described the reaction of pyridine, cyanogen bromide and an aromatic amine, which gave a yellow to a yellowish-green colour. It has been found that by buffering the solution at pH 6.1 by a phosphate buffer and determining the colour intensity by the extinction method, the extinction coefficient is directly proportional to the pyridine derivative concentration. The method is sensitive to a strength of 1 microgramme per ml.—H. Kringstaad and I. Naess, per *Nature, Lond.*, ii/1938, 1003.

The most satisfactory chemical method of determining the nicotinic acid content in various foodstuffs and tissues is that in which the pyridine nucleus is broken down by cyanogen bromide and aniline to give a yellow compound which is measured colorimetrically. Liver was found to contain 1.2 mg. per g. dry weight. Assuming a daily human requirement of 25 mg. nicotinic acid, it is concluded that $\frac{1}{4}$ lb. of fresh liver or $\frac{1}{4}$ lb. lean meat will suffice. In general, natural foods contain from 1 to 100 mg. of nicotinic acid per 100 g. dry material.—C. A. Elvehjem, per *Trop. dis. Bull.*, 1941, 398.

A photo-electric method based on the cyano-*p*-aminoacetophenone reaction has been applied satisfactorily to the determination of nicotinic acid in pharmaceutical products. By treating the alkali hydrolysates of materials containing the acid with acetone, substances, such as salts, proteins and coloured compounds, which interfere with photo-electric measurement are removed.—W. S. Jones, *J. Amer. pharm. Ass. (Sci. Edn.)*, 1941, 272.

A measured amount (up to 9 ml.) of aqueous solution (containing 0.005 to 0.25 mg. of nicotinic acid) is heated for 5 minutes at 75° to 80° and 1 ml. cyanogen bromide (4%) added, and placed on water-bath for 5 minutes. After cooling, 10 ml. of saturated aqueous solution metol (*p*-methylanilino-phenol sulphate) is added and diluted, to 20 ml. After standing at room temperature for 1 hour (excluded from light) the strength of the colour developed is measured in a Pulfrich photometer (filter S43) against a blank solution containing the same quantities of reagents. Claimed to be sensitive to 0.005 mg. with great accuracy.

In organic material containing nicotinic acid as a component of cozymase or Warburg's coferment, it must be liberated and the nicotinamide hydrolysed. The material (say 5 g.) is added to 10 ml. of 2N sodium hydroxide and kept in boiling water-bath for 30 minutes, cooled, and 1.8 ml. of hydrochloric acid (36%) added drop by drop and shaken (pH 4 to 6). After cooling, diluting to 20 ml. and standing for 20 minutes the mixture is centrifuged. 1 ml. of the clear liquid is vigorously shaken with 9 ml. of acetone and centrifuged for 3 to 4 minutes. 3 ml. of acetone layer and 3 ml. water are mixed and the acetone evaporated with a water vacuum pump. After transferring to a 20 ml. flask with N/15 potassium dihydrogen phosphate (volume not more than 9 ml.) the colour reaction is carried out in this practically clear, colourless solution.—Bandier and Hald, *Biochem. J.*, 33, 264.

The quantity of material containing about 0.4 mg. nicotinic acid can be suspended in 75 ml. of water, autoclaved 15 minutes at 10,500 kg. per sq. m. cooled, centrifuged, decanted and adjusted to 80 ml. After making alkaline with 20% sodium hydroxide, it is heated on steam-bath for 30 minutes to liberate the nicotinic acid from its amide, cooled and 2 ml. of 4% sodium bicarbonate solution followed by 1.5 ml. hydrochloric acid added, adjusted to pH 6.0 to 6.2 with 10% hydrochloric acid and diluted to 100 ml. 5 ml. in reaction tube, with 5 ml. of pH 6.0 potassium hydrogen phosphate-sodium hydroxide buffer solution is heated at 80° for 10 minutes; then 2 ml. of cyanogen bromide (sat. bromine water decolorised with 10% potassium cyanide) kept at 80° for a further 4 minutes; cool rapidly and after 4 minutes 0.2 ml. of *p*-aminoacetophenone solution (10% in 28 ml. of 10% hydrochloric acid with water to 100 ml.) added. After standing in the dark for 15 minutes and adjusting to 13 ml. the colour is extracted with ethyl acetate. Concentration of the colour complex is estimated in aliquot on the basis of the observed extinction values (Conning filters 038 and 511) against extinction values with 0, 20 and 40 mg. of added nicotinic acid, allowance being made for a blank without addition of cyanogen bromide.—Arnold *et al.*, *Industr. Engng Chem. (anal. Edn.)*, 1941, 62.

Above methods not suitable for starchy products unless submitted to diastasis.—Bina, Thomas and Brown, *Cereal Chemistry*, 1941, 661.

Phosphomolybdic acid suggested as a precipitant for nicotinic acid (2.5 g. phosphomolybdic acid warmed with 20 ml. 20% HCl and 20 ml. water, cooled, filtered and diluted to 50 ml). Aliquot containing 0.1 to 1.0 mg. nicotinic acid is treated with 0.2 to 0.3 ml. phosphomolybdic acid solution. Precipitate dissolved in 0.1N sodium hydroxide and reduced with 1 ml. of

stannous chloride in hydrochloric acid (10 g. in 25 ml.) and the colour produced by this reduction of the phosphomolybdic acid measured in a tintometer.—Daroga, *J. Soc. chem. Ind., Lond.*, 1941, 263.

A comparison is made of methods used for the extraction and assay of nicotinic acid from animal and plant tissues.—V. H. Childerin and R. R. Williams, *Industr. Engng Chem. (anal. Edn.)*, 1942, 671.

For determination of nicotinic acid in flour, see p. 818.

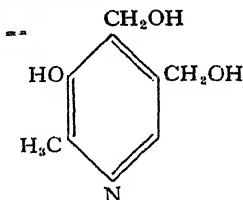
Nicotinamidum (B.P. Add. VI). $C_6H_6N_2O = 122.12$. Dried over sulphuric acid in a vacuum desiccator for 18 hours it loses not more than 0.5% and then contains not less than 98.5% of $C_6H_6N_2O$. Residue on re-ignition with sulphuric acid, not more than 0.1%. Arsenic limit, 2 parts per million. Lead limit, 10 parts per million. Assayed by hydrolysis with test solution of sodium hydroxide, distillation into excess N/10 sulphuric acid and back titration with N/10 sodium hydroxide using methyl red as indicator. Nicotinamidum, U.S.P. XII, complies with the same standards.

Tabellæ Nicotinamidi (U.S.P. XII). Contain 95 to 120% of the labelled amount of $C_6H_6N_2O$, including all tolerances. Assayed by refluxing 3 times a weighed quantity of powdered tablets with alcohol, filtering the alcohol after each extraction, collecting the insoluble residue on the filter and washing the flask and filter with alcohol, removing most of the alcohol, transferring the residual solution to a Kjeldahl flask, adding water and 30% sodium hydroxide solution, distilling into an excess of N/10 sulphuric acid and finally titrating the excess acid with N/10 sodium hydroxide using methyl red indicator.

Pyridoxine. Syn. VITAMIN B₆. $C_8H_{11}O_3N = 169.2$. Before its isolation in 1938 by Keresztesy and Stevens this vitamin was given a variety of names by different workers, including "factor Y," "factor I," vitamin H" and the "anti-dermatitis vitamin." Vitamin B₆, a factor necessary for the growth and well-being of pigeons, is also now thought to be the same substance.

Pyridoxine occurs naturally in cereals and seeds, yeast, rice bran, egg yolk, liver, etc., and may be prepared synthetically as 2-methyl-3-hydroxy-4:5 (hydroxymethyl)-pyridine. It is a white, odourless, crystalline powder, with a bitter taste, melting at 157° to 160°, with decomposition.

Pyridoxine has the formula:



The structure of vitamin B₆.—E. T. Stiller, J. C. Keresztesy and J. R. Stevens, *J. Amer. chem. Soc.*, 1939, 61, 1237; see also S. A. Harris, E. T. Stiller and K. Folkers, *ibid.*, 1242.

Synthetic vitamin B₆.—S. A. Harris and K. Folkers, *Science*, 1939, 89, 347; *ibid.*, *J. Amer. chem. Soc.*, 1939, 61, 3307.

Vitamin B₆ first became known as the "rat antidermatitis factor," but there has been some confusion between the condition produced by a lack of vitamin

B₆ and that produced by a lack of other members of the B complex. Chick *et al.* cleared up the position considerably and showed that rats deprived of vitamin B₆ only developed the characteristic dermatitis and, in time, a tendency to fits of an epileptic nature. Pure vitamin B₆ cured these rats.—*Biochem. J.*, 1940, 34, 580, 595.

In human nutrition vitamin B₆ has been found to relieve symptoms (extreme nervousness, insomnia, irritability, abdominal pain, weakness and difficulty in walking) in patients whose typically pellagrous and neuritic condition had been cured by nicotinic acid and vitamin B₁, but whose diet had remained unchanged and whose unhealthy condition had obviously been due to a lack of several factors.—T. D. Spies *et al.*, *J. Amer. med. Ass.*, ii/1939, 2414.

Six patients with pseudohypertrophic muscular dystrophy were greatly improved by injections of 50–500 mg. per week of vitamin B₆.—W. Antopol and C. E. Schotland, *J. Amer. med. Ass.*, ii/1940, 1058.

Determination of Pyridoxine. Since the discovery of vitamin B₆ several biological assay procedures for this factor have been proposed. Most of them have been based on the cure of acrodynia produced on a vitamin B₆-deficient ration or on a combination of growth and the cure of acrodynia of vitamin B₆-depleted rats. Colorimetric methods of assay have also been developed.

A biological estimation of pyridoxine based entirely on the growth response of vitamin B₆-deficient rats supplemented either with synthetic pyridoxine or the substance to be assayed.—T. W. Conger and C. A. Elvehjem, *J. biol. Chem.*, 1941, 138, 555.

Colorimetric determination of vitamin B₆. The vitamin reacts with 2,6-dichloroquinonechloroimide giving a blue colour.—J. V. Scudi, *J. bio. Chem.*, 1941, 139, 707.

The Denis phenol reaction and formation of carbopyridinium cyanin compound from the methyl ether methyl iodide of B₆. B₆ can be liberated from naturally occurring materials by pepsin digestion, removal of proteins with sodium tungstate and of purines, pyrimidines and amidazole bases by silver nitrate.—*Industr. Engng Chem. (anal. Edn.)*, 1941, 13.

Diazotised sulphanilic acid gives colour reaction with Vitamin B₆.—M. Swaminathan, *Nature*, 1940, 780.

Pyridoxine Hydrochloride. C₈H₁₁O₃N.HCl = 205.56. Pyridoxine is usually supplied in the form of the hydrochloride, which is prepared synthetically by a reaction between ethoxycetylacetone and cyanoacetamide. It occurs as a white odourless, crystalline powder with a saline taste and a melting-point of 206° to 208°. It is soluble 22 in 100 of water, 1.1 in 100 of alcohol (95%), and slightly soluble in other solvents.

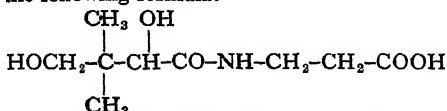
Stability of Pyridoxine Hydrochloride. It is reasonably stable to light and air. In solutions above pH 6 it decomposes, rapidly so on heating them; below pH 5 solutions are reasonably stable. Aqueous solutions have a pH of about 3. It is oxidised by strong oxidising agents and aqueous solutions are coloured red by ferric salts.

Pantothenic Acid. C₉H₁₇O₅N = 219.14. This factor, which was first isolated by R. J. Williams in 1938 from raw liver and which was found to possess the property of stimulating the growth of yeast, has been identified as the "chick anti-dermatitis factor." It has also been spoken of as the "liver filtrate factor," i.e., a factor present in the filtrate from liver extracts after various other factors (notably, vitamin B₁, riboflavin and pyridoxine) have been removed. It has also been found in similar yeast filtrates.

It is possible, however, that these filtrates contain other substances of a vitamin-like nature in addition to pantothenic acid. There is some evidence, though it is not yet definitely established, that "factor W," an additional nutrient required for the growth of the rat, and the "anti-grey-hair-factor," necessary to prevent greying of the hair of black rats, may be identified with pantothenic acid.

Pantothenic acid has been identified as a derivative of β -alanine, namely, $N-(\alpha\text{-}\gamma\text{-dihydroxy-}\beta,\beta\text{-dimethyl-butyl})\text{-}\beta'$ -alanine. It has been synthesised and is available in the form of its dextrorotatory calcium salt (calcium pantothenate).

It has the following formula:



Nature and partial synthesis of the chick antidermatis factor.—D. W. Woolley, H. A. Waisman and C. A. Elvehjem, *J. Amer. chem. Soc.*, 1939, 61, 977.

The structure of pantothenic acid.—R. J. Williams and R. T. Major, *Science*, 1940, 91, 246.

Improved synthesis of pantothenic acid.—D. W. Woolley, *J. Amer. chem. Soc.*, 1940, 62, 2251.

The total synthesis of pure pantothenic acid.—E. T. Stiller *et al.*, *J. Amer. chem. Soc.*, 1940, 62, 1785.

A deficiency of pantothenic acid in the diet of rats leads to nose-bleeding, a sticky exudate on the eyelids and depilation about the nose. Post-mortem examination revealed necrosis of the adrenals and some damage to the testes. The spleen and pancreas were apparently not affected.—Daft *et al.*, *Publ. Hlth Rep. Wash.*, 1940, 55, 1333.

It has not yet been shown that pantothenic acid is necessary for human nutrition. That it may be so was concluded from the effect obtained by injecting calcium or sodium pantothenate (in amounts up to 100 mg.) into 28 cases of beri-beri, pellagra and riboflavin deficiency in which the pantothenic acid content of the blood had been 20 to 50% lower than normal. Both the pantothenic acid and the riboflavin content of the blood were increased. Injection of large doses of riboflavin in cases of cheilosis raised the pantothenic acid content of the blood. Hence the function of pantothenic acid is probably associated with that of riboflavin.—T. D. Spies *et al.*, *J. Amer. med. Ass.*, 1/1940, 523.

Pantothenic acid is apparently closely allied with the cumulative effect of the vitamin B complex and has been shown to have a synergistic effect in the human system in association with riboflavin. Pantothenic acid, or its calcium salt, is to be considered a necessary constituent of all preparations intending to produce the therapeutic effect of the vitamin B complex.—*Amer. prof. Pharm.*, 1941, 298.

VITAMIN B₂

Chemistry of Vitamin B₂. Little is known of this factor beyond the fact that "something" occurring in dried yeast and wheat embryo is necessary to prevent loss of weight in pigeons fed on a diet of polished rice supplemented with liberal amounts of vitamin B₁.

Stability of Vitamin B₂. This factor is thermolabile.—*Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 167, 1932, pp. 165-6.

VITAMIN B₃

Chemistry of Vitamin B₃. This factor is found in bakers' yeast and can be separated from the watery extracts of vitamin B₁ by adsorption on Norite charcoal at pH 1.0. It is a base and forms a crystalline hydrochloride of the composition $\text{C}_4\text{H}_4\text{N}_4\text{HCl}_4\cdot\frac{1}{2}\text{H}_2\text{O}$. It is precipitated by phosphotungstic acid (pH 2.0 to 4.0), mercuric sulphate, picric acid, picrolonic acid and gold chloride. Pauly and nitroprusside tests are negative.—Kinnersley, O'Brien, Peters and Reader, *Biochem. J.*, 1933, 225.

Titration with 2 : 6-dichlorophenolindophenol shows a variation in ascorbic acid content of both fresh lemon and orange juices. The method has been used to test the value of preservatives and sterilisation. Fresh filtered lemon juice

pasteurised for 1 hour at 65° lost all its reducing power in 12 days when left exposed to air. The same juice treated with sulphur dioxide, instead of being pasteurised, completely lost its reducing power in 26 days when there was access of air. Sealed out of contact with air, the juice retains its activity very much longer when pasteurised or preserved with sulphur dioxide.—A. H. Bennett, *Analyst*, 1934, 91.

By the titration method it has been shown that the reducing power of orange juice is higher and more constant than lemon juice; and, in storage with preservatives to prevent fermentation, the reducing power may disappear completely in a few weeks. Acid and heat produce the same effect.—A. H. Bennett and J. D. Tarbert, *Biochem. J.*, 1933, 1294.

The ascorbic acid content of fruits and vegetables with special reference to the effect of cooking and canning.—M. Oliver, *J. Soc. chem. Ind., Lond.*, 1936, 153T. See also *Analyst*, 1938, 63, 2; *J. Soc. chem. Ind., Lond.*, 1941, 60, 586.

Sodium chloride inhibits the oxidation of ascorbic acid. Thus vegetables cooked in water containing sodium chloride retain their ascorbic acid better than those cooked in distilled water.—A. Hygaard and H. W. Rasmussen, *Nature, Lond.*, ii/1938, 293.

Biological Determination of Vitamin C. The biological assay of vitamin C must be carried out on guinea-pigs, for rats do not develop scurvy when fed on a diet deficient in vitamin C. The earliest signs of scurvy are seen in certain changes in the structure of the teeth. This can be used for the estimation of vitamin C. The changes have been carefully described by Höjer (*Acta paediatr., Stockh.*, 1924 (suppl.), and *Brit. J. exp. Path.*, 1926, 356). The extent to which the changes may take place in 2 weeks when guinea-pigs are fed on the scorbutic diet plus graded amounts of vitamin C have been described by Key and Elphick (*Biochem. J.*, 1931, 888), who also described a curve of response to vitamin C in the same paper.

The following are abbreviated accounts of the methods of assay given in the *B.P. Add. I*.

(a) *Tooth Method.* Guinea-pigs weighing from 250 to 300 g. receive a basal diet free from vitamin C for fourteen days. A suitable diet consists of:—

| | |
|---------------------------|-----|
| Bran | 45% |
| Split oats | 25% |
| Dried skimmed milk | 30% |

In addition each guinea-pig receives about 10 drops of a good sample of cod-liver oil twice a week.

For the experiment two groups are used, each of 10 guinea-pigs, one group receiving daily doses of the standard preparation and the other of the preparation being tested, for 14 days. A daily dose of the standard preparation is 1 mg. (20 units), and that of the preparation being tested an amount expected to contain the equivalent of 1 mg. The guinea-pigs are killed, the jaw-bones removed, decalcified, the roots of the incisor examined histologically, and the average degree of protection from scurvy of each group, as shown by the extent of disorganisation of the structure, is determined. The degree of protection may be represented by the figures 0 to 4, a moderate degree of protection being represented by the figure 2.5. If the average degree of protection of the two groups is equal then the activity of the

preparation is equal to that of the standard preparation. Otherwise, and if more exact information is required, the test is repeated, using the same dose of standard preparation for one group and for the other group a dose of the preparation being tested, which, judging from the first test, is likely to produce a degree of protection equal to that produced by the dose of the standard preparation.

Limits of Error. In an experiment in which the average effect (degree of protection from scurvy) is estimated for 10 guinea-pigs, the following statements can be made:—(1) There is no conclusive evidence of the presence of vitamin C unless the effect is greater than 1.6 units. (2) Two preparations can be shown to differ significantly in their activity only when their effects differ by more than 1 unit. (3) When the effect of each preparation is 2.5, the limits of error ($P = 0.99$) are 36 and 164%. When the effect of each preparation is 3.0 units, the limits of error ($P = 0.99$) are 51 and 149%.

(b) *Increase in weight method.* Young guinea-pigs weighing 250 to 300 g. receive unrestricted quantities of a diet free from vitamin C. A suitable diet consists of:—

| | | | | |
|--------------------|-----|-----|---|-----------------|
| Wheat bran | ... | ... | 6 | parts by volume |
| Barley meal | ... | ... | 2 | " " " |
| Wheat middlings... | ... | ... | 3 | " " " |
| Fish meal | ... | ... | 1 | " " " |
| Crushed oats | ... | ... | 4 | " " " |

In addition 40 to 60 ml. of autoclaved milk made up from dried powder is given to each guinea-pig every day. On this diet the guinea-pigs will develop scurvy and die in from 4 to 5 weeks. Groups of five guinea-pigs are given daily doses of 0.5 and 0.25 mg. of the standard preparation and of the preparation being tested, a group of five animals being used for each dose of the two preparations. In every group the daily dose is continued for not less than 42 days (preferably 60), and the animals weighed twice a week. The guinea-pigs are then killed and the signs of scurvy assessed. If the average growth and degree of protection from scurvy of the groups receiving the doses of the preparation being tested is equal or nearly equal to that of the other groups, the activity of the preparation is equal to that of the standard. Otherwise, and if more exact information is required, the test is repeated using fresh groups of guinea-pigs for the same dose of the standard preparation and other groups for doses of the preparation being tested, which, judging from the first test, are likely to produce a degree of protection equal to that produced by the doses of the standard preparation.

Limits of Error. In an experiment in which ten guinea-pigs receive the standard preparation and ten guinea-pigs receive the preparation being tested in a 6 weeks' test, and in which the dosage is just sufficient to maintain the mean weight constant, the limits of error ($P = 0.99$) are 82 and 139%. If the mean response is larger, the error is also larger.

Chemical Determination of Vitamin C. Ascorbic acid has strong reducing properties. Thus its concentration in fruit juices may be measured by means of a N/1000 (or M/2000) solution of 2 : 6-dichlorophenolindophenol. If ascorbic acid is present the solution is decolorised. The reaction is not specific, glutathione and cysteine giving the same reaction, but in juices which are known not to contain these substances, the reaction may replace the more laborious biological estimation. Solutions of potassium permanganate are also decolorised by ascorbic acid, forming a faintly brownish liquid. It also reduces silver nitrate solution, producing a black precipitate, and potassiocupric tartrate solution, producing a yellowish precipitate.

2 : 6-dichlorophenolindophenol does not measure dehydroascorbic acid or any non-reducing complex of ascorbic acid. The dehydroascorbic acid may be reduced and the non-reducing complexes be hydrolysed by a solution of stannous chloride in hydrochloric acid. Ascorbic acid is then destroyed by a treatment with ascorbic acid oxidase, obtained in the form of cucumber juice. Total ascorbic acid is obtained by the difference between titrations with indophenol before and after this enzyme treatment.

The reduced form only exists in vegetable tissue; it is oxidised by an oxidising enzyme to dehydroascorbic acid on exposure to air. This can be prevented by the addition of a strongly ionised acid to the extract which destroys the oxidising enzyme.—Mack and Tressler, *J. biol. Chem.*, 1937, 118, 735.

An assay method depending on a stable potassium iodate solution as the standard oxidant. At the same time use is made of the specific oxidation of ascorbic acid by 2 : 6-dichlorophenolindophenol. 2NHCl is added to 10 ml. citrus juice to give a solution of 0.1 to 0.5N. Indophenol dye (0.5 to 1.0 mg./ml.) is added until one drop of dye solution remains in oxidised form. Approximately 10 ml. of a 0.1N potassium iodide solution added and titration completed electrometrically with 0.1N potassium iodate (added drop by drop at intervals of 5 seconds). Another 10 ml. juice is treated with the iodate solution alone at the same acidity and iodide concentration, to give the total reducing capacity of the juice. The difference between the two figures gives a measure of the vitamin C content of the juice.—Ramsey and Colichman, *Industr. Engng Chem. (anal. Edn.)*, 1942, 319.

A direct titration with potassium iodate solution in the presence of acidified potassium iodide solution is adequate for the determination of ascorbic acid in citrus juices, as no interfering substance is present. End-point is said to be satisfactorily sharp.—Ballentine, *Industr. Engng Chem. (anal. Edn.)*, 1941, 13, 89.

Interference of tannin with the dichlorophenolindophenol method can be removed; tannin-containing drugs extracted with 5% trichloroacetic acid, adjusting to pH 3.8 with sodium acetate-acetic acid solution and tannin precipitated with lead acetate solution. If carried out rapidly, little oxidation of ascorbic acid occurs.—Mirimanoff and Toledo, *Quart. J. Pharm.*, 1941, 188.

Tannins in fresh and dried fruits removed by 5% trichloroacetic acid and precipitation as above, or by extraction by boiling for 4 to 5 minutes with 5% metaphosphoric acid.—Mirimanoff and Mori, *Quart. J. Pharm.*, 1941, 199.

A new procedure to eliminate the interference of a variety of extraneous substances in the assay of ascorbic acid has been worked out. The method depends upon the fact that ascorbic acid is rendered feebly reducing by reaction with formaldehyde at pH 3.5, whereas a number of interfering substances are rendered feebly reducing at pH 1.5. The assay is carried out under carefully controlled conditions at pH 1.5 and standard solution of 2 : 6-dichlorophenolindophenol is added in drops of definite size. The titration is continued to a persistence of limited time. Corrections are made for small amounts of un-titrated ascorbic acid present at the end-point.—J. W. H. Lugg, *Nature, Lond.*, ii/1942, 577.

The oxidation of ascorbic acid by copper is inhibited by NaCl, amino-acids and proteins.—E. M. Mystkowski, *Biochem. J.*, 1942, 36, 494.

Provided that the specified precautions are taken, direct titration of the acid extract against 2:6-dichlorophenolindophenol can be recommended with confidence for all ordinary routine analyses of plant materials, as giving the total antiscorbutic activity. Tables are given showing the close correspondence between the chemical and biological values.—L. J. Harris and M. Olliver, *Biochem. J.*, 1942, 36, 155.

Stable Solutions of 2:6-Dichlorophenolindophenol for use in determinations of ascorbic acid are prepared by distilling 500 ml. of dioxane, collecting the middle 400 ml. of distillate, and dissolving in this slightly more of the indicator than needed, adding 1 ml. of glacial acetic acid for every 100 ml. of solution, stirring for about 15 minutes and filtering. The filtrate is then standardised against ascorbic acid.—I. Stone, *Industr. Engng Chem. (anal. Edn.)*, 1940, 415.

Determination in Citrus Juices. Place 20 ml. of juice in a 250 ml. Erlenmeyer flask and add 4 ml. of 12N sulphuric acid to lower the pH to 0.02 to 0.08. Add 0.01N iodine solution until an excess of 1 to 2 ml. is present. Stand for about 30 seconds. Add 0.01N sodium thiosulphate solution in an excess of 1 ml., and 3 ml. of 0.5% starch solution as indicator. Finally, add 0.01N iodine until the end-point is reached. The total volume of iodine solution added minus the volume of iodine equivalent to the volume of thiosulphate solution equals the volume of iodine solution consumed by the reducing substances in the sample. 1 ml. of 0.01N iodine solution is equivalent to 0.88 mg. of ascorbic acid.—J. W. Stevens, *Industr. Engng Chem. (anal. Edn.)*, 1938, 269.

Determination in Tablets. The tablet is disintegrated by triturating in 2% acetic acid, and the mixture diluted to 500 ml. with acetic acid 2%, in a graduated flask. Five ml. are pipetted into a flask, mixed with 15 ml. of a 3% solution of trichloroacetic acid and the whole back-titrated with standard solution of 2:6-dichlorophenolindophenol until the rose colour produced remains for five seconds. The standard solution is prepared by dissolving 20 mg. of 2:6-dichlorophenolindophenol in 100 ml. of water, allowing to stand for 24 hours, filtering, and adding a little sodium bicarbonate to the filtrate.—E. C. M. J. Hollman, *Pharm. Weekbl.*, 1940, 77, 393.

Intradermal Test for Vitamin C Deficiency. The requisites are a sterile solution of 2:6-dichlorophenolindophenol, a 1 ml. tuberculin syringe graduated in hundredths, and an intradermal needle. The dye solution contains 2 mg. of the solid dye in 4.9 ml. of distilled water. The site chosen for injection is the skin on the forearm in an area free from hair and small superficial veins. The skin is cleaned with ether and stretched, the needle is inserted intradermally and 0.01 ml. of the solution injected under the epithelium, a wheal 2 mm. in diameter being raised. The times of injection and of complete disappearance of the dye colour are noted. In order to minimise any error which may arise in the size of the wheal, four wheals are raised and the average time for decolorisation taken. A decolorisation time of less than 5 minutes indicates tissue saturation with vitamin C, while 10 minutes or longer is in favour of a deficiency.—B. Portnoy and J. F. Wilkinson, *Brit. med. J.*, i/1938, 328.

Capillary Fragility Test. Subsistence on a diet markedly deficient in vitamin C gradually gives rise to fragility of the capillaries. On this has been based a fragility test to ascertain the presence of vitamin C subnutrition. The following method of carrying out the test is suggested by G. F. Göthlin, *Lancet*, ii/1937, 703.

The test consists in determining the number of petechiæ produced by the application of pressure to each arm under controlled conditions. With the patient lying down and arms relaxed a pressure of 35 mm. is applied by means of a suitable apparatus and the number of petechiæ visible through a 5-dioptre lens in a circular area 6 cm. in diameter within the folds of each arm is counted. After not less than 1 hour the number of petechiæ is counted again in case more have appeared during the interval, and the test is then repeated with a pressure of 50 mm. Since the number of petechiæ produced by the application of 35 mm. pressure is a more important indicator of fragility than the number produced by 50 mm. pressure, each petechia produced in the second test is counted as 1 and each produced in the first test is counted double. The total number produced in the two arms gives a double-sided petechial index. If it is expected that the investigation will have to be repeated within 3 weeks (for example, to ascertain the effect of treatment) the test must be carried out on one side only,

giving a one-sided petechial index. A double-sided index of more than 12 indicates abnormal fragility, but abnormality cannot be excluded with values of 9 to 12. For the one-sided test the corresponding values are 8 and 5 to 8 respectively.

The fragility test is suggested for use where facilities are not available for making ascorbic acid determinations in the blood and for mass investigations, e.g., in institutions.

A rapid method of differentiating children with large or small reserves of vitamin C.—J. Pemberton, *Brit. med. J.*, ii/1940, 217.

A test for vitamin C deficiency.—M. Vauthey, *Lancet*, i/1939, 695.

For further references to capillary fragility see Vitamin P, p. 766.

Excretion in health and disease. The diet has probably been unduly low in vitamin C when the daily excretion of ascorbic acid falls below 10 to 15 mg. per day per 10 stone body weight. The average daily excretion of normal adult subjects receiving small (not liberal) allowances of fruit (or other source of vitamin C) is about 20 mg. per day.—M. A. Abbasy, L. J. Harris, S. N. Ray, and J. R. Marrack, *Lancet*, ii/1935, 1399.

The absence of ascorbic acid in the urine indicates a shortage of vitamin C which should be corrected by giving large doses of this factor in the diet or by intravenous injection of ascorbic acid in cases where it cannot easily be taken orally.—L. J. Harris and S. N. Ray, *Lancet*, i/1935, 71; *ibid.*, 462.

On the Witwatersrand gold mines where the Bantu mine labourers work under hot, humid conditions, cases of scurvy and sub-scurvy occur with some frequency despite the fact that the diet provided has been shown to contain adequate amounts of vitamin C. It was found that appreciable amounts of vitamin C are eliminated in the sweat—the average weight loss of recruits undergoing a heat-tolerance test for 1 hour was $\frac{1}{2}$ lb., and as this represents chiefly sweat loss the excretion of vitamin C by this route amounts to some 2 mg. per hour. As a miner may lose from 2½ to 5 lbs. weight during an eight-hour shift underground, excretion of vitamin C through sweat must play an important part in vitamin C subnutrition in miners who cannot afford a liberal diet.—R. E. Bernstein, *Nature, Lond.*, ii/1937, 684.

A child should not be considered adequately nourished unless there is an abundance of vitamin C in the tissues, as judged by the overflow after a test dose of ascorbic acid.—J. Pemberton, *Brit. med. J.*, ii/1940, 217.

Osteomyelitis. In common with other infective conditions, osteomyelitis causes a diminished rate of excretion of vitamin C in the urine and a lowered response to test dose, indicative of an apparently increased usage of the vitamins during the infective process.—M. A. Abbasy, L. J. Harris, and N. G. Hill, *Lancet*, ii/1937, 177.

Pulmonary Tuberculosis. In patients with pulmonary tuberculosis the "deficit" in vitamin C shown by a lowered urinary excretion and a diminished response to test doses, is seen in an extreme form. There is a good correlation between the severity of the case as judged by the usual clinical standards and the diminution in the urine titres. Extra vitamin C should be given in pulmonary as in other forms of tuberculosis to make up for the wastage.—M. A. Abbasy, L. J. Harris, and P. Ellman, *Lancet*, ii/1937, 181.

Rheumatic Fever. Vitamin C deficiency is not an important factor in the causation of acute rheumatism though mild degrees of this deficiency are not uncommon in rheumatic children. The capillary resistance test is not a reliable guide to the deficiency.—C. B. Perry, *Lancet*, ii/1935, 426.

Rheumatoid Arthritis. A group of 25 patients with rheumatoid arthritis who had been kept on a standardised diet with a normally adequate allowance of vitamin C all fell below the standard in their excretion of the vitamin, a group of controls all being above standard.—M. A. Abbasy, L. J. Harris, and P. Ellman, *Lancet*, ii/1937, 181.

For details of methods of determination in urine and blood, see pp. 670 and 699.

VITAMIN D

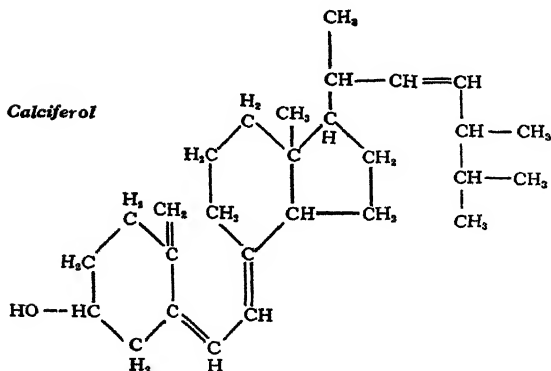
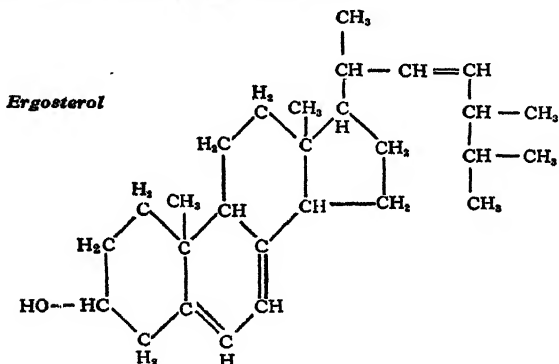
Chemistry of Vitamin D. The term vitamin D is applied to substances of the sterol group possessing antirachitic properties. There are probably at least ten such substances but only two are

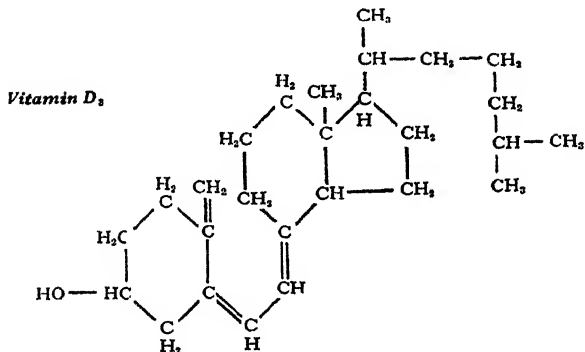
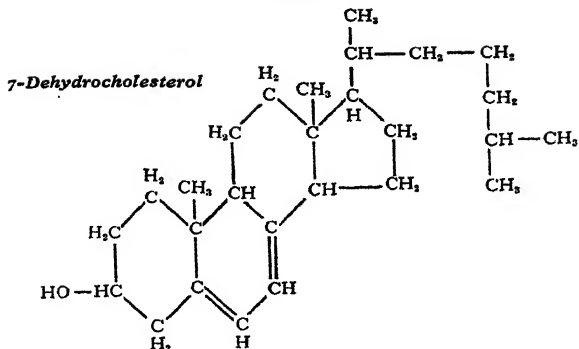
of therapeutic importance, namely, vitamin D₂ (calciferol), which is the product of the irradiation of ergosterol, a sterol obtained from yeast, and vitamin D₃ which occurs in fish-liver oils and may be prepared by irradiation of 7-dehydrocholesterol. There is no clear evidence that vitamin D₂ and vitamin D₃ differ appreciably in antirachitic activity in man.

The Chemistry of Vitamin D.—C. E. Bills, *J. Amer. med. Ass.*, ii/1938, 2150.

7-Dehydrocampesterol on irradiation with ultra-violet light yields an antirachitically active product. By comparison with ergosterol, irradiated under the same conditions, the antirachitic activity of vitamin obtained from 7-dehydrocampesterol has been estimated as 4,100,000 i.u. per gramme.—W. L. Ruigh, *J. Amer. chem. Soc.*, 1942, 1902.

Ring Structures of Ergosterol and Vitamin D.





Calciferol (*B.P. Add. I*). $C_{28}H_{44}O$ = 396.3. Contains in 1 milligram 40,000 units of antirachitic activity (vitamin D) when determined in relation to the standard preparation of antirachitic vitamin (vitamin D) by the biological assay. No precipitate of ergosterol is produced when a mixture of equal volumes of 1% *w/v* solution and a 1% digitonin solution in alcohol (90%) is allowed to stand for 12 hours. The ultra-violet absorption at 265 $m\mu$ is determined on a dehydrated alcohol solution, special precautions being taken.

An examination of the physico-chemical properties of 73 samples of calciferol, representing the result of carefully controlled and standardised conditions of manufacture, suggests that the following standards should be adopted: Melting-point, unsharp, 116°; $[\alpha]_{546.1}^{20}$ (4% *w/v* solution in alcohol) + 123.25° to + 125.75°; $E_{1\text{cm}}^{1\%}$ 265 $m\mu$, 460 to 500. The range for optical rotation allowed in *B.P. Add. I* is unnecessarily wide.—F. W. Anderson, A. L. Bacharach, and E. L. Smith, *Analyst*, 1937, 430.

Liquor Calciferolis (*B.P. Add. I*). Contains in 1 g. 3000 units of antirachitic activity (vitamin D) when determined by the biological assay of antirachitic vitamin (vitamin D) in relation to the Standard Preparation of antirachitic vitamin (vitamin D).

Liquor Vitamini D Concentratus (*B.P. Add. II*). Determined by the biological assay of antirachitic vitamin (vitamin D) in relation to the standard preparation of vitamin D, it contains in 1 g., 10,000 units of antirachitic activity (vitamin D) and not more than 5000 units of vitamin A activity. Acid value, not greater than 2.5.

Liquor Ergosterolis Irradiati (*B.P.*) contained in 1 g., 300 units of antirachitic activity, and was determined by the biological assay of antirachitic vitamin (vitamin D), but is now replaced by *Liquor Calciferolis*.

Oleovitamina D Synthetica (*U.S.P. XII*) is assayed by the revised assay for vitamins A and D, and the method of activation must be stated on the label. Contains in 1 g. not less than 10,000 U.S.P. units of vitamin D.

Stability of Calciferol. Crystalline calciferol dissolved in olive oil, halibut-liver oil (2 samples), cod-liver oil (2 samples) or in liquid paraffin and stored in nitrogen filled bottles at 0° for 15–20 months suffered little loss in vitamin D activity. Halibut-liver oil (3 samples) and cod-liver oil (2 samples) stored at 0° and cod-liver oil (3 samples) stored at room temperature also suffered very little loss in antirachitic activity.—H. M. Bruce, E. W. Kassner and G. E. Phillips, *Quart. J. Pharm.*, 1938, 11, 46.

Biological Determination of Calciferol. The following is an abbreviated account of the method suggested in the *B.P.* '32:—

(a) *Curative.* Select from 3 or 4 litters about 20 young rats ranging in weight from 50 to 60 grammes, and feed for 3 weeks on a rachitogenic diet, e.g.:

| | | | |
|---------------------|--------|---------------------|--------|
| Ground yellow maize | 33% | or | |
| Whole wheat | .. 33% | Ground yellow maize | 76% |
| Wheat gluten | .. 15% | Wheat gluten | .. 20% |
| Gelatin | .. 15% | Calcium carbonate | 3% |
| Calcium carbonate | 3% | Sodium chloride | .. 1% |
| Sodium chloride | .. 1% | | |

Divide the rats into two groups, distributing the rats of each litter as evenly as possible, the rats in one group receiving daily doses of the standard preparation, e.g., 0.25 to 1.0 unit, and those in the other, doses of the preparation being tested. Different rats receive different daily doses, but one rat receives the same dose on each day and the doses are continued for 10 to 14 days. The rats are killed and the extent to which the rickets has been cured estimated by X-rays or by examination of the bones after staining (remove the distal ends of the ulnæ and radii, immerse for 24 hours in 4% w/v aqueous solution of formaldehyde, cut in halves by longitudinal section, immerse in 1.5% w/v aqueous solution of silver nitrate for a few minutes and expose to light).

The degree of healing produced by a given dose is not the same in every rat, therefore the average effect of a dose of the preparation being tested in a group of rats should be compared with the average effect of a dose of the Standard Preparation in another group. Where these effects differ, information as to the activity of the preparation being tested, in terms of the Standard Preparation, can be gained from the results, provided the average effect in groups of rats of a series of different doses of the Standard Preparation has been previously determined, the difference being determined from the difference between the average effect produced by the preparation being tested and that produced by the Standard Preparation.

(b) *Prophylactic.* About 20 young rats, weighing 40 to 50 grammes, from 3 or 4 litters, are fed on a rachitogenic diet for 4 or 5 weeks. During this period they are divided into two groups (as in (a)), one group receiving daily doses of the preparation being tested and the other daily doses of the Standard Preparation in a dose of about 0.1 unit, a given daily dose of either preparation being given to each of not less than 5 rats. At the end of the period the rats are killed and corresponding bones taken from each rat. Moisture and fat are removed from the bones, which are then weighed, incinerated in a crucible, the weight of ash determined, and the percentage of ash in the dry extracted bone calculated. The average percentage of ash in the bones of rats receiving the same doses is then calculated. A dose of the preparation being tested producing the same average percentage of ash as that produced in another group of bones by a known dose of the Standard Preparation is compared in activity with the latter and the activity expressed in units. Where the average percentage of ash in the bones of rats differs between those receiving the preparation being tested and those receiving the Standard Preparation, the test is repeated, using doses of the preparation being tested such as may be judged to produce an average percentage of ash the same as that produced by known doses of the Standard Preparation.

Limits of Error. In an experiment in which ten rats receive the Standard Preparation and ten rats receive the preparation being tested, and the litters are evenly divided between the two groups, the limits of error ($P = 0.99$) are 59 and 170%.

Absorption Spectrophotometry. The absorption spectrum of calciferol has a maximum at 265 $m\mu$ between the maxima at 260 and 270 $m\mu$ given by ergosterol, so that it is not possible to use absorption spectrophotometry to estimate vitamin D₂ in the presence of ergosterol. 7-Dehydrocholesterol and 22-dihydroergosterol can also function as provitamin D and also produce inaccuracies in the spectrophotometric determination of vitamin D. Interference may also be due to the presence of vitamin A. The solution of the problem may lie in separating vitamin D from the interfering substances by the absorption technique employed in chromatographic analysis.

Colorimetric Determination of Vitamin D. A deep violet colour is produced on the addition of pyrogallol and aluminium chloride in alcoholic solution to solution of vitamin D. The solution of sterols in benzene, light petroleum or chloroform is evaporated to 0.25 ml. and 5 to 10 drops of 0.1% solution of pyrogallol in dehydrated alcohol are added. The mixture is heated on a water-bath,

2 to 4 drops of freshly prepared 10% solution of anhydrous aluminium chloride in dehydrated alcohol are added and the heating continued. In the presence of vitamin D a deep violet colour appears, reaching a maximum in 5 minutes. The reaction products are dissolved in dehydrated alcohol, dry carbon dioxide is blown over the surface and the tube closed with a rubber stopper. The colour of the solution (lilac red) is compared with that produced by similarly treating known amounts of vitamin D. The optimum amount for colorimetric determinations is 0.01 to 0.1 mg., but as little as 0.002 mg. can be detected. Oily solutions of the vitamin must be saponified, the solution evaporated *in vacuo* and the test carried out on a light petroleum extract dried with anhydrous sodium sulphate. The reagent is not affected by cholesterol, ergosterol or lumisterol, but suprasterol II affects it although to a smaller extent than calciferol.—W. Halden and H. Tzoni, *Nature, Lond.*, i/1936, 909.

Colour tests have been proposed for vitamin D, using a solution of antimony trichloride in anhydrous chloroform. This gives, with vitamins D₂ and D₃, a pale yellow colour or yellowish-pink, if a trace of acetyl chloride is present. Vitamin A, if present, must first be removed by treatment with maleic anhydride.—F. Wokes, *Chem. & Drugg.*, ii/1942, 428-9.

Vitamin D₃. Chemical and biological tests have identified the crystalline vitamin D₃ obtained from tunny-fish liver oil with that obtained by the irradiation of 7-dehydrocholesterol. The substance has a melting-point of 82° to 84° and an absorption maximum at 265 mμ. Tested biologically on rats it has been found to have a potency of 40,000 i.u. of vitamin D per mg. It is highly active for chickens, and the British Standards Institution has issued a method for the biological assay by the chick method (*B.S.S. No. 911*).

Windaus, Lettne and Schenck prepared 7-dehydrocholesterol and activated it by irradiation.—*Liebigs Annalen der Chemie*, 1935, 520, 98.

Vitamin D potencies of concentrates of liver and body fats from 16 different species of fish and from two mammalian sources were identical. The vitamin D from fish livers differs from the vitamin D₂ in irradiated ergosterol in ultra-violet absorption, optical rotation and behaviour on esterification with phthalic anhydride.—O. Kygh, *Nature, Lond.*, ii/1935, 396, 552.

Brockmann prepared a crystalline 3 : 5-dinitrobenzoate of the vitamin from tunny-liver oil, and recovered a colourless non-crystalline hydrolytic product. He proposed the name D₃ for the vitamin.—*Hoppe-Seyl. Z.*, 1936, 241, 100.

Windaus, Schenck and v. Werder have isolated the crystalline 3 : 5-dinitrobenzoate of the active irradiation product as well as its colourless, non-crystalline hydrolytic product. Both appear to be identical with the products from tunny-liver oil.—*Hoppe-Seyl. Z.*, 1936, 241, 100.

Chemistry of calciferol and vitamin D₃—a summary.—A. L. Bacharach, *Nature, Lond.*, ii/1936, 387.

Some irradiation products from 7-dehydrocholesterol.—A. Windaus, M. Deppe and W. Wunderlich, *Liebigs Annalen der Chemie*, 1937, 533, 118.

The 3 : 5-dinitrobenzoate of vitamin D was prepared from Japanese blue-fin tunny oil. The vitamin prepared from the ester was compared with the International Standard and found to contain 35,000 i.u. per mg. Another preparation was found to contain 40,000 i.u. per mg. By chemical tests it was found to be identical with the vitamin D formed by the irradiation of 7-dehydrocholesterol.—H. Brockmann and A. Busse, *Hoppe-Seyl. Z.*, 1937, 249, 176, and *Naturwissenschaften*, 1938, 26, 122.

The production of an antirachitic provitamin from cholesterol.—N. A. Milas and R. Heggie, *J. Amer. chem. Soc.*, 1938, 60, 984.

Relative Potency of Vitamin D from Different Sources. Irradiated ergosterol is markedly inferior to cod-liver oil as a source of vitamin D for chickens when given in equal vitamin dosage as determined on rats. The general weight of evidence favours a figure of from 10 to 20 : 1 as the relative potency for chickens. Ergosterols from yeasts and from ergot, the yolk of eggs of hens fed with large amounts of irradiated ergosterol and the milk of cows fed on irradiated yeast or irradiated ergosterol are all inferior to cod-liver oil, in the same rat dosage, for chickens. Irradiated milk is equal to cod-liver oil. There

is a chemical difference in the vitamin from the two sources. Several other well-authenticated forms of vitamin D have been prepared in the laboratory and it is more than probable that the vitamin as present in natural sources is not always the same chemical substance. It seems fairly certain that for the human infant vitamin D from fish-liver oils, irradiated ergosterol, irradiated cholesterol and vitamin D milks of various kinds have the same relative potency as for rats. For human use, therefore, standardisation of sources of vitamin D against irradiated ergosterol as standard, using rats as the test animals, is satisfactory, but for use with poultry standardisation must be carried out with chickens only. At present only the vitamin D from fish-liver oils is of practical interest for poultry feeding.—E. M. Hume, *Nutr. Abstr. Rev.*, 1937, 6, 891.

Summary of investigations on relative potency of different sources of vitamin D for different species.—A. L. Bacharach, *Food*, 1937, 6, 180.

Efficacy of calciferol (vitamin D_3) and the vitamin D of cod-liver oil, possibly vitamin D_2 , for human infants. These are equal in antirachitic potency for human infants.—Eliot *et al.*, *J. Pediatr.*, 1936, 9, 357. See also N. Morris and M. M. Stevenson, *Lancet*, i/1939, 876.

The comparative efficiency of vitamin D from cod-liver oil and irradiated cholesterol for laying birds.—R. M. Bethke *et al.*, *Poultry Science*, 1937, 16, 438.

The relative antirachitic potencies of vitamin D_2 and vitamin D_3 . A summary of experiments organised for the Accessory Food Factors Committee of the League of Nations. It was concluded that vitamin D_2 and vitamin D_3 have for the rat the same antirachitic potency, namely, 40,000 i.u. per mg.—*Bull. Hlth Org. L. o. N.*, 1940-41, 9, 434.

For vitamin D content of fish oils, see pp. 289 and 769.

VITAMIN E

Chemistry of Vitamin E. This is an oil-soluble substance occurring in wheat-germ oil and other natural sources. When the non-saponifiable fraction of wheat-germ oil is removed there remains a fraction having the characteristic physiological action of vitamin E. From this fraction α -tocopherol, a compound having marked vitamin E activity, has been isolated.

Tocopheryl Acetas (B.P.C. Supp. III). $C_{55}H_{102}O_2 = 472.6$. Tocopheryl acetate is the acetate of natural α -tocopherol or of synthetic racemic α -tocopherol and occurs as a pale yellow, almost odourless, viscous fluid. It contains not less than 96% and not more than the equivalent of 100% of total tocopherol calculated as the acetate, $C_{55}H_{102}O_2$. Specific gravity, 0.954 to 0.966. Refractive index at 20°, 1.4958 to 1.4972. Ultra-violet absorption in dehydrated alcohol at 285.5 m μ , about 42. 2 drops dissolved in 1 ml. light petroleum and shaken for 1 minute with 1 ml. distilled water of pH about 5.5, gives a separated aqueous layer of pH 5.0 to 6.5. A test for limit of uncombined tocopherol is included in which a blue-violet colour should be produced when 0.05 ml. of diphenylamine solution and 0.2 ml. of N/100 ceric sulphate solution are added to a solution of 0.02 g. in 1 ml. of dehydrated alcohol.

Synthetic racemic α -tocopheryl acetate has been adopted as the international standard for vitamin E. The international unit is defined as the specific activity of 1 milligram of the standard preparation (the average amount which, administered orally, prevents absorption-gestation in rats deprived of vitamin E).

The international standard is issued in the form of a solution in olive oil, of which 0.1 g. contains 1 i.u.

Stability. It is stable at high temperatures in the dry state and is not destroyed by cooking. It is stable in light except after prolonged exposure to ultra-violet light. Synthetic racemic α -tocopheryl acetate in oil as used in experimental work prior to the adoption of an international standard was found to be stable over several months but wheat-germ oil should never be kept longer than eight months. Tocopheryl acetate is not stable to oxidising agents and is easily destroyed by rancid fats and ferric chloride.

Biological Determination. The biological estimation of vitamin E is based on the mean fertility dose determined on rats, i.e., that dose which enables 50% of the rats used to bear a live litter.

Criteria of response determined on female rats as follows:—At autopsy on the 16th day the uterus is removed and weighed, both before and after removal of living foetuses, dead foetuses and resorption sites. Numerical values for what may be designated the "uterine index" are obtained:—

$$\frac{\text{wt. of uterine contents (in grammes)} + \text{no. of viable foetuses (when 2 or more are present)}}{5}$$

Values less than unity represent negative responses, of which those less than 0.35 are classed as "completely negative" while those greater than 0.35 but less than 1 are termed "subminimal." Positive responses range from 1 to 4 and are generally proportional to the size of the dose.—K. E. Mason, *Vitamin E Symposium, Soc. Chem. Ind.*, 1939.

Mean fertility doses compared.—A. L. Bacharach, *Nature, Lond.*, ii/1938, 35.
International Standard for vitamin E.—*Bull. Hlth Org., L. o. N.*, 1940-41, 9, 436.

Chemical Determination. Heat about 0.25 g. of tocopheryl acetate under a reflux condenser with 7 ml. of dehydrated alcohol until solution is complete. Add 5 ml. of a 12% v/v mixture of sulphuric acid and dehydrated alcohol and boil for three hours. Cool, transfer to a 25 ml. graduated flask and make up to volume with dehydrated alcohol. To 10 ml. of the resulting solution add 2 drops of diphenylamine solution and titrate with N/100 ceric sulphate solution by means of a micro-burette until the colour changes to blue-violet. Each ml. of N/100 ceric sulphate solution is equivalent to 0.002362 g. of $C_{55}H_{102}O_6$.

The Emmerie and Engel method uses ferric chloride in alcoholic solution in the cold as oxidising agent and determines the ferrous salt thus formed with α - α' -dipyridyl colorimetrically. Reaction colour is reasonably stable and readings made after 10 to 15 minutes. Blank must always be run and freshly prepared solutions of ferric-chloride-dipyridyl must be used. (The colour of the blank solution increases on standing, but is overcome by working in a dark room with artificial light only.—Lester, Smith and Bailey.) The reaction is also given by vitamin A and the carotenoids. Separation effected in benzene solution by absorption on purified Floridin XS earth.—Emmerie and Engel, *Vitamin E Symposium, Soc. Chem. Ind.*, 1939.

Estimation of the intensity of absorption at 294 $m\mu$ is only suitable for pure or nearly pure specimens of synthetic tocopherol—many other substances (e.g., those present in wheat-germ oil concentrates) also show absorption at 294 $m\mu$. The Karrer and Kelby method oxidises tocopherol with alcoholic gold chloride at about 50°, following the reaction electrometrically; equilibrium only established slowly on account of low concentration of oxidising agent present.—Lester, Smith and Bailey, *Vitamin E Symposium, Soc. Chem. Ind.*, 1939.

Spectroscopic estimations may be applied to vitamin E concentrates when chromatographic absorption is useful in removing difficulties. Small concentrations of vitamin E may be estimated by potentiometric titration using gold chloride as oxidising agent, making suitable allowance for interference by carotenoids.—F. Wokes, *Chem. & Drugg.*, ii/1942, 430.

Estimation in Wheat-Germ Oil and Products. Emmerie and Engel claim that determination can be carried out, but Lester, Smith and Bailey state that assays must be done on the unsaponifiable fraction of the oil. Emmerie and Engel report good agreement between biological and chemical methods, whereas Lester, Smith and Bailey state that physical and chemical methods cannot distinguish between α - and β -tocopherols, which may differ in biological activity. Using the nitric acid oxidation method after chromatographic fractionation on activated alumina, it has been shown that 55% of vitamin E activity is located in the embryo of the wheat grain, the remainder being distributed throughout the remainder of the berry.—Birmingham and Andrews, *Cereal Chemistry*, 1941, 678.

It has been proposed that treatment with 85% sulphuric acid will remove carotenoids and other substances that interfere with the iron-dipyridyl method for the determination of tocopherol. A petroleum spirit extract of wheat-germ oil is treated with 85% sulphuric acid, centrifuged and the supernatant layer washed with dilute alkali—the thus purified solution reduces ferric iron immediately.—Parker and McFarlane, *Canad. J. Res.*, 1940, 405.

VITAMIN F

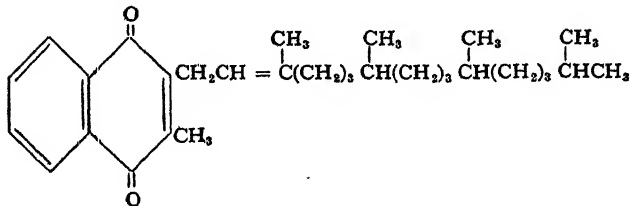
This was the name originally given to a factor thought to be specially needed for lactation, but the idea has now been discredited and the name dropped. It was then suggested as a designation for the antineuritic vitamin when vitamin B was first thought to consist of at least two factors, but it is now scarcely used by anyone in this connection.

More recently it has been employed as a name for certain unsaturated fatty acids in whose absence "scaly tails" develop in rats. As the identity of these compounds, linolic and linoleic acids, was recognised as soon as their effect was recognised, it seems unnecessary to give them the indeterminate name "vitamin". Claims for the good effect of "vitamin F" on the human skin (in face creams, etc.) must be unfounded as no one could possibly take so little of these unsaturated fatty acids in their diet as to derive benefit from the small amount contained in face creams. The effect of the deficiency in rats is only obtained by most stringent removal of all unsaturated fatty acids from their diet.

VITAMIN K

Chemistry of Vitamin K. Two vitamin K substances have been obtained from natural sources, vitamin K_1 from alfalfa grass, a light yellow oil which crystallises on cooling in acetone or alcohol solution, and vitamin K_2 from putrefied fish meal, a light yellow crystalline solid. Vitamin K_1 , 2-methyl-3-phytyl-1:4-naphthaquinone, is probably the most active.

It has the formula:



Vitamin K activity and structure.—L. F. Fieser, M. Tishler and W. L. Sampson, *J. biol. Chem.*, 1939, 137, 659.

Synthesis of vitamin K₁.—L. F. Fieser, *J. Amer. chem. Soc.*, 1939, 61, 3467.

Constitution and synthesis of vitamin K₁.—D. W. MacCorquodale *et al.*, *J. biol. Chem.*, 1939, 131, 357.

Isolation of vitamin K₂.—R. W. McKee *et al.*, *J. biol. Chem.*, 1939, 131, 327.

Menaphthone (*B.P. Add. VI*). $C_{11}H_8O_2 = 172.2$. Menaphthone is 2-methyl-1:4-naphthaquinone and is a synthetic therapeutic equivalent of vitamin K₁. When dried in a vacuum desiccator over sulphuric acid it loses not more than 0.2% and then contains not less than 98.5% of $C_{11}H_8O_2$. Ash, not more than 0.05%; m.p., 105° to 107°. The presence of chromium is limited by a test in which the substance is incinerated and the residue fused with a mixture of anhydrous potassium carbonate, anhydrous sodium carbonate and sodium peroxide (1.75 : 1.35 : 1). The residue is dissolved in water, the solution acidified and treated with solution of diphenylcarbazide and the violet colour produced compared with that given by a standard solution of potassium dichromate similarly treated. In the assay, anhydrous sodium carbonate and sodium potassium tartrate solution are added to a solution of the substance in alcohol and acetic acid, which is then titrated with N/10 titanous chloride solution in an atmosphere of carbon dioxide, potassium indigodisulphonate solution being used as indicator.

Menadionum (*U.S.P. XII*). Dried over sulphuric acid, loses not more than 0.3% and then complies with the same standard. Ash, not more than 0.1%; m.p., 105° to 107°. In the assay, the substance, dried over sulphuric acid in the dark, is dissolved in glacial acetic acid and hydrochloric acid, zinc dust added and the mixture allowed to stand in the dark for 30 minutes in a stoppered flask bearing a Bunsen valve. The solution is decanted through cotton wool and the flask washed with recently boiled and cooled distilled water. The solution and washings are titrated against N/10 ceric sulphate solution, using orthophenanthroline as indicator. A blank titration is performed and the necessary correction made.

Tabellæ Menadioni (*U.S.P. XII*). Contain 95 to 115% of the labelled amount of $C_{11}H_8O_2$, including all tolerances. Assayed by extracting a weighed quantity of powdered tablets with chloroform, removing the chloroform, dissolving the residue in glacial acetic acid, adding dilute hydrochloric acid and zinc powder and allowing to stand in a flask carrying a Bunsen valve for 20 minutes or until the solution is colourless. After filtration through a pledget of cotton the solution is titrated with N/10 ceric sulphate using orthophenanthroline indicator and allowance is made for a blank determination performed on the reagents.

Acetomenaphthone (*B.P. Add. VI*). $C_{15}H_{14}O_2 = 258.1$. Acetomenaphthone is 1:4-diacetoxy-2-methyl-naphthalene, prepared by reducing 2-methyl-1:4-naphthaquinone with zinc and acetic acid in the presence of acetic anhydride. It melts between 112° and 114°, and loses on drying at 80° not more than 1%. A limit test for zinc is included.

Biological Determination of Vitamin K. Several groups of investigators have devised bio-assay procedures for vitamin K. In general, the procedures are either curative or preventive; in the former the deficiency is produced by maintaining young chicks on a diet devoid of vitamin K. After the deficiency has been produced in a severe form the substance to be tested is administered, and

after a definite time interval, a sample of blood is taken for the determination of clotting time. In normal chicks the clotting time is less than three or four minutes; in chicks with a severe degree of deficiency it is over two or three hours. In these deficient chicks treated with the proper quantity of vitamin K the clotting time is restored to a normal value within a period of six hours.

Various units for vitamin K activity have been proposed and the best known of these may be expressed in relation to weight as 2000 Ansbacher units, or 1700 Thayer-Doisy units, or 25,000 Dam units, all of which equal the potency of 1 mg. of 2-methyl-1:4-naphthaquinone.

An assay procedure based on a modification of the short curative method described by Ansbacher (*J. Nutrition*, 1939, 17, 303) and similar to the 18-hour method of Thayer and Doisy (*Proc. Soc. exp. Biol. N.Y.*, 1939, 40, 478; *ibid.*, 41, 194). Day-old chicks were fed on a vitamin K-free diet for 12 days and their blood-coagulation time then determined. When 90% of the birds showed coagulation times of 60 minutes or more the entire group was considered ready for assay purposes. The material to be assayed was dissolved in pea-nut oil and the solution diluted with the same solvent to such an extent that the quantity of substance to be fed was always contained in 0.1 ml. of the solution. After administration of the dose into the crop the birds were kept for 18 hours without food and the blood-coagulation time then determined. The criterion for the effective dose was the minimum amount of material which, when administered in 0.1 ml. of pea-nut oil, reduced the clotting times of 60 to 80% of the vitamin K-deficient birds to less than 10 minutes in the 18-hour period. It was found that with the large majority of chicks 0.3 γ of 2-methyl-1:4-naphthaquinone met the criterion for the effective dose, or 1 γ of pure vitamin K₁ (synthetic). These results indicate that 2-methyl-1:4-naphthaquinone is 3.3 times as potent as the vitamin in the chick assay.—L. F. Fieser, M. Tishler and W. L. Sampson, *J. biol. Chem.*, 1939, 137, 659.

Chemical Determination of Vitamin K. Dissolve about 0.2 g. of menaphthone in a mixture of 10 ml. of alcohol (95%) and 15 ml. of glacial acetic acid. Add 4 g. of anhydrous sodium carbonate and 25 ml. of a 10% solution of sodium potassium tartrate and titrate with N/10 titanous chloride solution in an atmosphere of carbon dioxide, 3 drops of a 0.1% potassium indigodisulphonate solution being used as indicator. Each ml. of N/10 titanous chloride solution is equivalent to 0.008603 g. of C₁₁H₈O₂.

A colorimetric method capable of application to tablets and ampoules is described for estimating quantities of the order of 0.5 mg.—J. L. Pinder and J. H. Singer, *Analyst*, 1940, 7.

Two methods are described. The first is an oxidation-reduction method: To about 0.1 g., accurately weighed, of the sample previously dried over sulphuric acid for three hours, add 10 ml. of glacial acetic acid and 10 ml. of hydrochloric acid 10%. When the menaphthone has dissolved, add about 1 g. of zinc dust, close the flask with a Bunsen valve and allow to stand in subdued light for half an hour, shaking occasionally. Then dilute the solution with oxygen-free water, decant quickly through cotton, washing the flask and cotton with three 10 ml. portions of oxygen-free water, and titrate the filtrate promptly with N/10 ceric sulphate, using 0.1 ml. of orthophenanthroline as indicator; each ml. of N/10 ceric sulphate is equivalent to 0.008609 g. of C₁₁H₈O₂. The method is also applicable to tablets, which are first powdered and then extracted with chloroform, the chloroform evaporated off and the residue treated as above. The second method is a bromination method:—About 0.1 g., accurately weighed, is placed in an iodine flask and dissolved in 5 ml. of carbon tetrachloride. 25 ml. of N/10 bromine are added, followed by 2 ml. of hydrochloric acid, and the flask stoppered, shaken for five minutes and allowed to stand for one hour with occasional shaking. 10 ml. of 10% potassium iodide solution are added

and the flask washed down with 25 ml. of water. The liberated iodine is titrated with N/10 sodium thiosulphate; each ml. of N/10 bromine is equivalent to 0.008609 g. of $C_{17}H_{15}O_2$. A blank experiment, omitting the menaphthone, should be performed.—J. Rosin *et al.*, *Amer. J. Pharm.*, 1941, 73, 434.

Vitamin K and associated quinones and naphthaquinones can be estimated by reduction to the corresponding hydroquinone and back-titration to the quinone with 2:6-dichlorophenolindophenol. Method and apparatus described.—N. R. Trenner and F. A. Bachner, *J. biol. Chem.*, 1941, 137, 745.

For quantitative tests for prothrombin concentration in blood, see p. 680.

VITAMIN P

Szent-Györgyi and his co-workers (Armentano *et al.*) reported in 1936 the presence in extracts of Hungarian red pepper and, later, in lemon juice, of a substance other than ascorbic acid which could control the number of hæmorrhages occurring in the course of certain conditions by increasing the resistance of capillary walls to the application of pressure. They called this fraction vitamin P or citrin. Citrin was later found to consist of mixed crystals of two different substances, one hesperidin (m.p. 261°) forming the major part, and the other an eriodictyol glucoside to which the activity of citrin was attributed. Recently, R. H. Higby (*J. Amer. Pharm. Ass., Sci. Edn.*, 1943, 74) investigated crude preparations of the flavanone constituents of citrus peel, including orange hesperidin, lemon citrin and lemon eriodictin, all of which were found to contain, in varying proportions, both the blood pressure reducing factor and the capillary permeability factor. It was further shown that crude orange hesperidin containing no eriodictyol (present, in addition to hesperidin, in both citrin and eriodictin) was active in reducing blood pressure while pure hesperidin and pure eriodictyol were inactive owing to their insolubility. The active substance in crude orange hesperidin was shown to be hesperidin chalcone, an unstable yellow pigment easily reverting to the insoluble hesperidin. Methylation of the chalcone, by preventing this reversion, causes it to become permanently water-soluble and preliminary tests indicate that this methyl chalcone affords protection against capillary fragility and is effective in lowering blood pressure though further tests are necessary to determine possible toxicity.

Rich sources of vitamin P include oranges, lemons, grapes, plums and prunes. Moderately good sources include grape-fruit and a puree made of rose-hips. Poor sources include tomatoes and green vegetables.—H. Scarborough, *Chem. & Ind.*, 1941, 618.

Blackcurrants contain vitamin P; a water-soluble concentrate prepared from blackcurrants was 100 times as active as recrystallised hesperidin.—A. L. Bacharach and M. E. Coates, *Analyst*, 1942, 317.

A biological test for vitamin P employing guinea-pigs.—A. L. Bacharach *et al.*, *Biochem. J.*, 1942, 36, 407.

Purpura hæmorrhagica after arsenic therapy successfully treated with vitamin P.—D. R. Gorrie, *Lancet*, i/1940, 1105.

The erythema and dermatitis occurring as toxic manifestations of anti-syphilitic therapy are shown to be associated with a low capillary resistance and clinical improvement follows the use of vitamin P.—G. Horne and H. Scarborough, *Lancet*, ii/1940, 66.

A deficiency of vitamin P may exist in man even when he has been taking large doses of ascorbic acid for long periods. Its clinical manifestations include pains in the legs and across the shoulders, weakness, lassitude and fatigue, with

a reduced capillary resistance characterised by the development of spontaneous petechial hæmorrhages. It responds to treatment with vitamin P. Description of six cases.—H. Scarborough, *Lancet*, ii/1940, 644.

Hesperidin has been administered to patients whose capillary tonus was decreased in vascular hæmorrhagic diseases as a result of trauma, pressure, avitaminosis, bacterial invasion, chemical injury or lymphatic infiltration. A large measure of success was attained where the purpura was allergic (2 cases), infective (1 case), or nutritional (1 case), but not where it was mechanical.—I. N. Kugelmass, *J. Amer. med. Ass.*, i/1940, 519.

VITAMIN REQUIREMENTS OF MAN

A very great deal of work has been done to determine the daily vitamin requirements of man, but the only pronouncements of any authoritative collected opinion are the minimum amounts considered necessary by the League of Nations Health Organisation (*Quart. Bull.*, 1938, 7, 475) and the optimal amounts suggested by the National Nutrition Conference for Defense, U.S.A. 1941 (*Publ. Hlth Rep., Wash.*, 1941, 56, 1233). These are summarised below:—

| For a man of 70 kilos | Vit. A i.u. | Vit. B ₁ i.u. | Vit. C mg. | Vit. D i.u. | Ribo- flavin mg. | Nico- tinic Acid mg. |
|--------------------------|----------------|-----------------------------|---------------|----------------|------------------------|-------------------------------|
| Minimum requirements | 3000 | 300 | 30 | — | — | — |
| Optimal requirements | 5000 | * { 700 600 500 | 75 | — | * { 3.3 2.7 2.2 | * { 23 18 15 |

* According to heavy, medium or light work.

There is no evidence of harmful effects from overdosage of any of the vitamins except perhaps vitamin D and then only when the diet contains excessive amounts of calcium. Even then the ill effects can be counteracted by taking large amounts of salads and green vegetables.

The best way to ensure adequate intake of vitamins (both known and unknown) is to eat "whole" foods or foods which have been processed as little as possible. If a food is "refined" in some way, a valuable part of it may be removed, and this part will almost certainly contain not only known substances but unknown substances, some of which may be essential for perfect nutrition. Vitamin concentrates should only be resorted to when serious deficiency is suspected, for even the best of them can only make up certain deficiencies, not all.

The following tables contain vitamin values of various foods. It should be realised that different samples vary greatly in their vitamin content. Moreover, in assessing the vitamin content of a diet, it should be remembered (a) that vitamin A is affected very little by cooking but that vitamins B₁ and C may be partly dissolved

out in the cooking water (about one quarter) and partly destroyed by heat (about one quarter), hence the vitamin B₁ and C contents of foods may be reduced to one-half by ordinary processes of cooking; and (b) it is not certain that man can make full use of the carotene in vegetables and therefore the vitamin A value of a vegetable to man is probably only about a half or one-third of that indicated by a chemical determination of its carotene content.

AVERAGE VITAMIN CONTENT OF FOODS

Expressed as International Units or Milligrams
per 100 Grammes

The following are *average* values only. The range may be from one-half to twice each value given, or even greater.

| | Vitamin A Content | | | Vit. B ₁ Int. units per 100 g. | Ribo- flavin mg. per 100 g. | Vit. C mg. per 100 g. | Vit. D Int. units per 100 g. |
|-----------------------------------|--|--|----------------------------------|---|---|--------------------------------|--|
| | Int. units per 100 g. biol. tests | Spectro. determin- ation × 1600 Int. units per 100 g. | Caro- tene γ per 100 g. | | | | |
| Cereals | | | | | | | |
| Arrowroot .. | Nil | — | — | — | 0.01 | — | — |
| Barley (whole grain) .. | Nil | — | — | 100 | — | 5 (soaked) | — |
| Bread— white .. | Nil | — | — | 20 | 0.05 | — | — |
| wholemeal .. | — | — | — | 120 | — | — | — |
| National wheatmeal | Nil | — | — | 110 | — | — | — |
| Oatmeal (porridge) .. | Nil | — | — | 150 | 0.02 | — | — |
| Rice— polished .. | Nil | — | Nil | Nil | 0.05 | — | — |
| unpolished | Nil | — | 34 | 70 | 0.07 | — | — |
| Sago (dry) .. | — | — | — | Nil | — | — | — |
| Dairy | | | | | | | |
| Produce | | | | | | | |
| Butter, fresh | 4000 | — | 480 | Nil | 0.008 | — | 60 |
| Cheese, Cheddar .. | 1200 | — | — | 10 | 0.17 | — | 50 |
| Cream .. | — | — | — | — | — | — | — |
| Egg— white .. | Nil | — | — | 150 | 0.4 | Nil | — |
| yolk .. | 1500 | — | — | 100 | 0.5 | Nil | — |
| Milk, fresh— whole .. | 300 | — | — | 20 | 0.2 | 1.5 | — |
| skimmed .. | Nil | — | — | 20 | — | 1.8 | — |
| Meat, Poultry and Game | | | | | | | |
| Bacon .. | Nil | — | — | 200 | — | Nil | — |
| Beef .. | — | — | 60 | 50 | 0.2 | Nil | — |
| Chicken .. | Nil | — | — | 60 | 0.05 | — | — |
| Ham .. | — | — | — | — | — | — | — |

| | Vitamin A Content | | | Vit. B ₁ Int. units per 100 g. | Ribo- flavin mg. per 100 g. | Vit. C mg. per 100 g. | Vit. D Int. units per 100 g. |
|--|--|--|----------------------------------|---|---|--------------------------------|--|
| | Int. units per 100 g. biol. tests | Spectro. determin- ation × 1600 Int. units per 100 g. | Caro- tene γ per 100 g. | | | | |
| Meat, Poultry and Game (continued) | | | | | | | |
| Kidney— | | | | | | | |
| Sheep .. | — | — | — | 190 | — | — | — |
| Ox .. | — | — | — | — | 1.5 | — | — |
| Liver, calf .. | 16,000 | — | — | 100 | 1.8 | — | Nil |
| Mutton, leg | 50 | — | — | 80 | 0.27 | 2.5 | — |
| Pork, loin, | | | | | | | |
| lean and fat | Nil | — | — | 180 | 0.1 | 1.9 | — |
| Rabbit .. | Nil | — | — | 10 | 0.07 | 1.0 | — |
| Sweetbreads | — | — | — | 106 | — | 11.0 | — |
| Veal, fillet .. | — | — | — | 36 | 0.07 | — | — |
| Fish | | | | | | | |
| Cod .. | — | — | — | 30 | 0.2 | — | — |
| Haddock, | | | | | | | |
| fresh .. | — | — | — | 20 | 0.16 | — | — |
| Halibut .. | — | — | — | 40 | 0.15 | — | — |
| Herring .. | 4000 | — | — | 20 | 0.25 | — | — |
| " roe .. | — | 500 | — | — | 0.45 | — | — |
| Mackerel .. | — | — | — | 30 | 0.66 | — | — |
| Oysters .. | 420 | — | — | — | 0.46 | 3.0 | 5 |
| Salmon, fresh | — | — | — | — | — | 150.0 | 500 |
| Shrimps .. | — | — | — | — | 0.16 | — | — |
| Trout .. | — | — | — | — | 0.68 | — | — |
| Fish Oils | | | | | | | |
| Cod, flesh oil | — | — | — | — | — | — | 5000 |
| Herring, | | | | | | | |
| flesh oil .. | — | 5000 | — | — | — | — | 12,000 |
| Mackerel, | | | | | | | |
| flesh oil .. | — | — | — | — | — | — | 7000 |
| Salmon, | | | | | | | |
| flesh oil .. | — | 400 | — | — | — | — | 15,000 |
| Sardine, | | | | | | | |
| body oil .. | — | 100,000 | — | — | — | — | 8000 |
| Shrimp, | | | | | | | |
| flesh oil .. | — | — | — | — | — | — | 6000 |
| Tuna, | | | | | | | |
| flesh oil .. | — | — | — | — | — | — | 7000 |
| Fish, Liver Oils | | | | | | | |
| Cod .. | — | 100,000 | — | — | — | — | 10,000 |
| Haddock .. | — | 10,000 | — | — | — | — | 1000 |
| Halibut .. | — | 6,000,000 | — | — | — | — | 250,000 |
| Hake .. | — | 500,000 | — | — | — | — | 13,000 |
| Mackerel .. | — | 12,000,000 | — | — | — | — | 200,000 |
| Salmon .. | — | 200,000 | — | — | — | — | 130,000 |
| Sardine .. | — | — | — | — | — | — | 11,000 |
| Shark .. | — | 800,000 | — | — | — | — | 2000 |
| Sturgeon .. | — | 2,000,000 | — | — | — | — | 0 |
| Tuna .. | — | 5,000,000 | — | — | — | — | 4,000,000 |

| | Vitamin A Content | | | Vit. B ₁ Int. units per 100 g. | Ribo- flavin mg. per 100 g. | Vit. C mg. per 100 g. | Vit. D Int. units per 100 g. |
|-------------------------|--|--|----------------------------------|---|---|--------------------------------|--|
| | Int. units per 100 g. biol. tests | Spectro. determin- ation × 1600 Int. units per 100 g. | Caro- tene γ per 100 g. | | | | |
| Fruit | | | | | | | |
| Apples— | | | | 40 | 12.0 | 2.6 | — |
| Eng., eating | — | — | — | — | — | 16.0 | — |
| Bramley, seedling .. | — | — | — | — | — | 10.0 | — |
| Apricots, dried .. | — | — | 5000 | — | 0.06 | 8.0 | — |
| Bananas .. | — | — | 124 | 50 | 0.01 | 10.0 | — |
| Cherries .. | — | — | — | — | — | — | — |
| Currants, black .. | 400 | — | — | 10 | — | 180.0 | — |
| Dates .. | — | — | 600 | 30 | 0.03 | — | — |
| Gooseberries .. | — | — | — | — | — | 40.0 | — |
| Grapefruit .. | — | — | 12 | 40 | — | 45.0 | — |
| Grapes, black .. | — | — | 15 | — | 0.005 | 2.0 | — |
| Lemons .. | Nil | — | — | 10 | 0.003 | 50.0 | — |
| Melon, cantaloupe .. | — | — | — | 15 | 0.065 | 35.0 | — |
| Oranges .. | — | — | 350 | 40 | 0.01 | 50.0 | — |
| Peaches .. | — | — | 760 | 25 | 0.01 | 7.0 | — |
| Pears .. | — | — | 80 | 30 | 0.02 | 3.0 | — |
| Pineapple .. | — | — | 160 | 25 | 0.07 | 25.0 | — |
| Plums .. | — | — | 100 | 40 | 0.03 | 5.0 | — |
| Prunes .. | — | — | — | 80 | — | 1.0 | — |
| Raisins .. | — | — | — | 75 | — | — | — |
| Raspberries .. | — | — | — | 30 | — | 30.0 | — |
| Rose hips .. | 8000 | — | — | Nil | 0.01 | 400.0 | — |
| Strawberries .. | — | — | — | — | — | 55.0 | — |
| Nuts | | | | | | | |
| Almonds .. | — | — | — | 80 | — | 10.0 | — |
| Chestnuts .. | — | — | — | 90 | — | 40.0 | — |
| Cob nuts .. | — | — | — | 200 | — | 15.0 | — |
| Coconut, fresh .. | — | — | Nil | — | 0.1 | 5.0 | — |
| Peanuts .. | — | — | 63 | 200 | 0.32 | 10.0 | — |
| Walnuts .. | — | — | 1000 | 150 | — | 30.0 | — |
| Vegetables | | | | | | | |
| Asparagus .. | — | — | — | — | — | 40.0 | — |
| Beans— | | | | | | | |
| butter .. | — | — | — | 160 | — | — | — |
| runner .. | 600 | — | — | 100 | — | 8.0 | — |
| Beetroot .. | — | — | Nil | 70 | — | 6.0 | — |
| Brussels sprouts .. | — | — | — | 60 | — | 100.0 | — |
| Cabbage .. | 900 | — | — | 55 | 0.05 | 80.0 | — |
| Carrots .. | 8000 | — | — | 60 | 0.02 | 5.0 | — |
| Cauliflower .. | — | — | 38 | 110 | — | 60.0 | — |
| Celery .. | — | — | 6000 | Trace | — | 3.0 | — |
| Cucumber .. | — | — | Nil | 30 | — | 8.0 | — |
| Leeks .. | — | — | — | — | — | 15.0 | — |
| Lettuce .. | — | — | 2000 | 90 | — | 6.0 | — |
| Marrow .. | — | — | Nil | — | — | 10.0 | — |

| | Vitamin A Content | | | Vit. B ₁ Int. units per 100 g. | Ribo- flavin mg. per 100 g. | Vit. C mg. per 100 g. | Vit. D Int. units per 100 g. |
|----------------------------------|--|--|-----------------------------|---|---|--------------------------------|--|
| | Int. units per 100 g. biol. tests | Spectro. determin- ation × 1600 Int. units per 100 g. | Carotene γ per 100 g. | | | | |
| Vegetables (continued) | | | | | | | |
| Onions .. | — | — | 25 | 40 | — | 9.0 | — |
| Parsley .. | — | — | — | — | — | 280.0 | — |
| Parsnips .. | — | — | 30 | — | — | 25.0 | — |
| Peas .. | — | — | 140 | 70 | 0.08 | 24.0 | — |
| Potatoes .. | — | — | Nil | 40 | 0.01 | 15.0 | — |
| Radishes .. | — | — | 3 | 30 | — | 16.0 | — |
| Spinach .. | — | — | 4000 | 40 | 0.06 | 60.0 | — |
| Tomatoes .. | 2300 | — | — | 40 | 0.06 | 30.0 | — |
| Turnips .. | — | — | — | 40 | — | 30.0 | — |
| Watercress .. | — | — | — | 18 | — | 55.0 | — |
| Beverages | | | | | | | |
| Cocoa powder | — | — | — | Nil | — | — | — |
| Coffee (bean) | — | — | — | 140 | — | 56.0 | — |
| Marmite .. | — | — | — | 800 | 3.3 | — | — |
| Beers | | | | | | | |
| Pale ale | — | — | — | 3 | — | — | — |
| Mild ale | | | | | | | |
| Strong ale | | | | | | | |
| Stout .. | — | — | — | — | 0.03 | — | — |
| Yeast | | | | | | | |
| Fresh .. | — | — | — | 82 | — | — | — |
| Dried brewers' .. | — | — | — | 2000 | 2.5 | — | — |

GENERAL REFERENCES TO VITAMIN CONTENT OF FOODS

Tables of vitamin content of human and animal foods.—M. A. Boas Fixsen and M. H. Roscoe, *Nutr. Abstr. Rev.*, 1938, 7, 823; 1940, *ibid.*, 9, 795.

The vitamin content of human foods as affected by processes of cooking and canning.—M. A. Boas Fixsen, *Nutr. Abstr. Rev.*, 1938, 8, 281.

The Vitamin Chart.—L. B. Pett, *Canad. med. Ass. J.*, i/1941, 181.

Vitamin content of food.—*Brit. med. J.*, ii/1940, 456.

Antiscorbutic values of fruits and vegetables.—M. Olliver, *Lancet*, ii/1940, 190.

Vitamin C content of wild fruit products.—M. Andross, *Analyst*, 1941, 358.

Tables are given stating the vitamin contents of meat and offals. Details of mineral content of meat are also given.—A. L. Bacharach, *Chem. & Ind.*, 1942, 405.

Vitamin content of vegetables.—M. Pyke, *J. Soc. chem. Ind., Lond.*, 1942, 10, 149.

Distribution of vitamin B₁ in 190 foods.—M. Pyke, *J. Soc. chem. Ind., Lond.*, 1939, 338.

MILK AND MILK PRODUCTS

MILK ANALYSIS

The importance of milk as an article of diet is reflected in the following pages by the number of Acts, Regulations and Orders by which its production and distribution are controlled, and by the various methods, chemical, physical and bacteriological, which are enlisted in its examination. While it is an ideal food when pure, especially for the young and growing, and for invalids, it is also an excellent medium for the development of organisms, both beneficial and harmful, and in the latter case it provides effective means for their distribution throughout a neighbourhood. If, by careless handling, a milk supply becomes contaminated with the germs of disease, it may lay the foundation of future illness in the individual or it may cause and spread an epidemic.

Probably no food has been more subjected to analysis and study than milk and the various products derived from it. It is a very complex fluid containing milk-fat in the form of an emulsion, proteins (casein, albumin, etc.) dissolved or in colloidal suspension, the carbohydrate lactose, and salts of organic and inorganic acids, together with minute proportions of many other substances including enzymes and vitamins.

In the accompanying table are mean figures, taken from Leach's *Food Inspection and Analysis*, which show the relative proportions of the main constituents in milk from different sources.

| | Cow | Human | Goat | Ewe | Mare | Ass |
|------------------|--------|-------|--------|--------|--------|-------|
| Specific gravity | 1.0315 | 1.03 | 1.0305 | 1.0341 | 1.0347 | 1.036 |
| Water % .. | 87.27 | 87.41 | 85.71 | 80.82 | 90.78 | 89.64 |
| Casein % .. | 3.02 | 1.03 | 3.20 | 4.97 | 1.24 | 0.67 |
| Albumin % .. | 0.53 | 1.26 | 1.09 | 1.55 | 0.75 | 1.55 |
| Fat % .. | 3.64 | 3.78 | 4.78 | 6.86 | 1.21 | 1.64 |
| Milk Sugar % .. | 4.88 | 6.21 | 4.46 | 4.91 | 5.67 | 5.99 |
| Ash % .. | 0.71 | 0.31 | 0.76 | 0.89 | 0.35 | 0.51 |

The average percentage composition by weight of cows' milk is given in the following table:—

| | | | | | |
|----------|-----|-----|-----|-----|-------|
| Water | ... | ... | ... | ... | 87.31 |
| Fat | ... | ... | ... | ... | 3.67 |
| Sugar | ... | ... | ... | ... | 4.78 |
| Ash | ... | ... | ... | ... | 0.73 |
| Protein | ... | ... | ... | ... | 3.42 |
| Casein | ... | ... | ... | ... | 2.86 |
| Albumin | } | ... | ... | ... | 0.56 |
| Globulin | | | | | |

The average percentage of total solids may be taken as 12.69, and of solids-not-fat as 9.02.

Legal Standard. The Sale of Milk Regulations, 1939, provide that a sample of milk that contains less than 3% of fat or less than 8.5% of other solids is to be presumed for the purposes of the Food and Drugs Act, 1938, not to be genuine until the contrary is proved. A sample of separated milk (machine-skimmed milk) containing less than 8.7% of solids-not-fat is similarly presumed not to be genuine until the contrary is proved. Thus the onus of proving such samples to be genuine is put on the vendor.

Although these limits are substantially lower than the average percentages of genuine milk, it is recognised that a proportion of genuine milk falls below one or both of the limits. It may be said that the data at present available are insufficient to permit of any exact estimate of this proportion.

That the risk of such a deficiency is appreciable may be gathered from the following experimental evidence. This is given in the form of a table of results obtained by Cranfield between 1923 and 1926 at the Midland Agricultural College from the analysis of weekly samples of the milk of 15 herds. The samples were taken from the mixed milk of the whole herd in each case, the herds including as large a variety in size, etc., as possible.

Of the 730 samples of mixed milk 59, or 8.1%, contained less than 3% of fat; and of 518 samples of milk 60, or 11.6% contained less than 8.5% of solids-not-fat.

Tocher found that of 626 samples of milk of individual cows 56, or 8.3%, contained less than 3% of fat, and 167, or 24.7%, contained less than 8.5% of solids-not-fat.

Other figures quoted include analyses of milk taken from churns delivered to two dairy companies over a period of one year. From these it is concluded that 7 to 8% is a maximum figure for the percentage of total churn samples likely to be found deficient in fat, and 5% a similar maximum figure in the case of non-fatty solids, for milks sold by dairy farmers of this class.

Similar figures have been given by Bailey (*J. Ass. off. Agric. Chem., Wash., 1922*) and by Monier-Williams (*Rep. Dep. Comm. Milk and Cream Regs., 1901*).

The percentages of fat and solids-not-fat in commercial milk depend upon several factors, one of the most important being the number of cows whose mixed milk is being sold. In large cities, where processed milk is almost exclusively retailed, samples of genuine milk giving figures falling below the minimum requirements of the Sale of Milk Regulations are practically unknown.

Natural Variations in the Composition of Milk.

Bulletin No. 16, published by the Ministry of Agriculture and Fisheries in 1932 under the above heading, includes the work of several observers and summarises the position of our knowledge on the subject to that date. The most important variations include:

The breed of the cow. The kind of milk yielded by cows of different breeds varies greatly, and in general the heaviest milkers give the lowest percentages of total solids. Jersey and

Guernsey cows give milk of high total solids and exceptionally rich in fat.

Age of the cow. The yield of milk increases until the twelfth year; the percentages of both fat and solids-not-fat decrease slowly with increasing age.

Variations during milking. The first-drawn milk is lowest in fat, the percentage increasing in successive drawings; the strippings are richest in fat. In taking "appeal-to-the-cow" samples, the cow must be completely milked.

Morning and evening milk. Cows are milked twice daily at unequal intervals; the greater the interval the larger the volume of milk yield and the smaller the percentage of fat. The solids-not-fat remains the same.

Time of the year. The yield of both fat and solids-not-fat varies continuously, being lowest in summer and highest in winter.

Period of lactation. Percentages of fat and of solids-not-fat both tend to fall until the fourth month and then to rise to the end of the period.

Kind and quality of food. Numerous experiments have proved that the percentage yield of both fat and solids-not-fat is not appreciably altered by varying either the amount or nature of food; only the yield is affected.

Abnormal conditions. Some cows are affected by unusual conditions. Change of temperature, change of environment, sexual excitement, change of milker, are examples of conditions causing changes in the composition of milk, and frequently such changes include considerable lowering of the percentage of fat.

Individual cows. Some apparently healthy cows yield milk falling persistently below the legal standard. Such milk usually varies from day to day more widely than that from normal cows.

Analysis of Milk.

The total solids and fat may be determined directly or the specific gravity at 60°F. and fat may be determined, the total solids calculated from the formula

$$T = 0.25G + 1.2F + 0.14$$

where T equals total solids, F equals fat, and G equals excess of gravity over 1.000; or Richmond's Milk Scale based on this formula may be used. Such a formula can only be accurate if the percentage composition of the components of the solids-not-fat remains the same. Vieth's ratio, lactose: protein: ash = 13:9:2, is approximately correct for most samples of milk. Samples widely differing in composition are classed as abnormal milks, and in these cases the formula given above does not hold.

(1) *Specific Gravity.* A small specially designed hydrometer (lactometer) may be used. If the temperature of the milk is not widely different from 60°F. the sp. gr. at that temperature is taken and converted to the sp. gr. at 60°F. using Richmond's Milk

Scale for the purpose. To obtain the sp. gr. from the lactometer reading, add 1000 and divide by 1000. The sp. gr. may be more accurately determined by using a sp. gr. bottle of 50 ml. capacity, bringing the temperature of the sample to 60° before weighing.

(2) **Total Solids.** Evaporate 5 g. of the sample in a tared stainless steel engine-turned dish on a boiling water-bath for an hour, dry in a water-oven for two hours, cool and weigh. If the milk is at all acid some volatile matter in addition to water will be driven off and the residue may tend to char. Neutralise with N/10 barium or strontium hydroxide to phenolphthalein, deducting the weight of barium or strontium added from the weight of the residue.

(3) **Fat.** Many different processes are in use for this important determination and of these the Werner-Schmidt and Gottlieb methods are convenient for rapid estimations, but a modified Adams extraction method is preferred when greater accuracy is required.

Gottlieb Method. 10 ml. of milk is shaken with 1 ml. of 0.96 ammonia, 10 ml. of alcohol and 25 ml. of ether. Light petroleum, 25 ml., is added, the contents of the tube well mixed, and from the clear layer of about 50 ml. an aliquot portion is removed, evaporated and weighed. As in the Werner-Schmidt process, it is preferable to draw off the ethereal layer by means of wash-bottle tubes, and repeat the extraction twice more, or until all the fat is removed. Recover the solvent and weigh the residue. Dissolve the fat in light petroleum and deduct the weight of the insoluble matter, if any.—*cf. Analyst*, 1927, 408.

Extraction Process (Adams). 5 ml. of sample is run from a pipette on a strip of "fat-free" filter paper and allowed to dry. This is then rolled up, dried at 100°, and extracted with pure dry ether for 5 hours in a Soxhlet apparatus. The ether is distilled off and the fat weighed, after drying at 100° for about 20 minutes.

Werner-Schmidt Method. Take 10 ml. of the milk in a 50 ml. tube, graduated in tenths of a ml., and 10 ml. of hydrochloric acid, and boil with shaking until the liquid turns dark brown. Cool rapidly in water and add 30 ml. of ether. Shake vigorously and allow to separate. Read off volume of ether and by means of a pipette transfer 10 ml. to a tared beaker. Evaporate ether, dry at 100°, and weigh. In preference, for accurate work, exhaust the contents of the tube with several quantities of ether and weigh the whole.

Gerber Process. This is a rapid method employed in routine work where several samples have to be examined and the results agree closely with those obtained by the Gottlieb and Adams methods. The determinations are carried out in special bottles called butyrometers, which are provided with a graduated stem ending in a conical bulb. 10 ml. of sulphuric acid (sp. gr. 1.820–1.825) is run into a butyrometer from an automatic pipette, followed by 11 ml. of the milk and 1 ml. of amyl alcohol run in carefully down the side of the bottle so as not to allow the contents to mix. The rubber stopper is inserted and the contents well mixed by shaking and inverting to include the contents of the stem until the whole is dark-brown in colour. The bottles thus prepared are immersed in water at 80° for eight minutes, then centrifuged for three minutes at 1000 revolutions per minute, and finally allowed to remain in water at 65° for a further 8 minutes. The volume of the fat is read from the graduated stem to 0.05°, giving the percentage directly. By moving the stopper slightly the column of fat may be brought into a suitable position for the reading.

When milk has been preserved with formaldehyde, the reaction mixture is violet in colour.

Homogenised milks require special treatment. The sp. gr. should not be determined until some hours have elapsed after procuring the sample. The times of heating the samples during the fat determinations should be increased somewhat and a second centrifuging and re-heating may be found necessary.

The fat in machine-skimmed milk can be determined by the Gerber method, using precision butyrometers made for the purpose. Total solids are calculated

from the sp. gr. and fat determinations, and the figure for solids-not-fat thus obtained.

B.S.S., No. 696, 1936.—*Part I* describes the apparatus and *Part II* the methods required for accurate routine purposes by the Gerber method. The specification includes the determinations of fat in milk, skim milk, separated milk, buttermilk, cream, cheese, and dried milk.

Minor modifications to *Part I.*—*Analyst*, 1941, 338.

Should the fat or solids-not-fat fall below or near to the legal standard, further determinations are necessary.

Maceration Method. T. E. Thorpe's method for sour milk. See *Analyst*, 1905, 197, and 1906, 317.

(4) **Ash.** Evaporate 25 g. in a tared platinum dish. Ignite carefully so as not to volatilise the chlorides.

(5) **Lactose (Milk Sugar).** The percentage content falls during the lactation period, but is greater with increased yield of milk.

The sugar can be determined by a volumetric or gravimetric estimation of its copper-reducing power after removal of proteins and fat. For gravimetric purposes this is best effected by diluting 25 ml. of the milk to about 400 ml. in a 500 ml. graduated flask and adding 10 ml. of Fehling's No. 1 copper solution and 35 ml. of N/10 sodium hydroxide. After shaking thoroughly and adjusting to the mark the liquid is filtered through a dry paper, 50 ml. of the filtrate being used for the determination. For a volumetric process with Fehling's solution see the method of Lane and Eynon under Condensed Milk. It is not necessary to clarify the milk, and the process is quick and accurate.

Another convenient and rapid process is that of H. D. Richmond (*Analyst*, 1925, 17). 10 g. of milk is weighed into a 100 ml. graduated flask, diluted with 50 ml. of distilled water and 10 ml. of Mayer's reagent and 2 ml. of N/1 sulphuric acid added. The whole is well shaken, diluted to 100 ml. and filtered. After neutralising 25 ml. of the filtrate to phenolphthalein (1 drop used), 20 ml. of N/10 iodine solution and 30 ml. of N/10 caustic soda solution are added. The mixture is allowed to stand for 20 minutes and after the addition of 4 ml. of N/1 sulphuric acid the excess of iodine is titrated with N/10 sodium thiosulphate.

Percentage of lactose ($C_{12}H_{22}O_{11}H_2O$)

$$= \text{ml. N/10 iodine used} \times 0.072 \times \frac{100 - (0.3 + \text{fat} \times 1.1)}{\text{weight of milk taken}}$$

Lactose Determination by Polarimeter. Add to 60 ml. of the milk 10 ml. of a solution of mercury in twice its weight of nitric acid (sp. gr. 1.43) diluted with four times its volume of water. Make volume up to 102.4 ml., filter. Note rotation in 200 mm. tube—divide by 2 and by 53, the specific rotation for lactose. Result is the amount of lactose per ml. in the solution. Multiply by 100 to give the amount in 60 ml. of milk.

Low Solids-not-Fat. Genuine samples of milk low in solids-not-fat are invariably low in lactose and high in chloride content. The maintenance of the osmotic pressure of such milk at a normal level is effected by the substitution of ionised chloride for lactose. Thus Vieth's Ratio is unaffected by the addition of water to milk, but is upset in genuine milk having low solids-not-fat, the proportion of ash being raised and of lactose lowered. Determination of the freezing-point of milk (see page 778) is the most valuable test we possess to distinguish between genuine milk of low solids-not-fat and watered milk.

(6) **Protein.** Protein may be determined by the method of Kjeldahl, using the factor 6.38 to convert nitrogen to protein.

Amino-Acid Number. To 10 ml. of sample add 1 ml. of 0.5% phenolphthalein solution and neutralise with N/10 strontium hydroxide. Add 2 ml. of 40% formaldehyde and continue the titration until neutral. Deduct the acidity of the formaldehyde solution from the second titration and calculate the difference to mls. of N. alkali per litre of sample. The figure is converted into proteins by multiplying by 0.170.

Casein. Ten g. of the sample is diluted with 40 ml. of water at 40°, 1.5 ml. of 10% acetic acid added and the mixture stirred gently. After standing for 20 minutes, 4.5 ml. of 0.25N sodium acetate is added, the mixture gently stirred and allowed to stand for an hour. The precipitated casein is filtered through a fluted filter-paper, washed with distilled water or with sodium acetate plus acetic acid solution of the strength used in the precipitation. The filter-paper and curd are transferred to a Kjeldahl flask and the amount of nitrogen determined in the usual way. $N \times 6.38 = \text{casein}$.

Lactoglobulin. Ten g. of the sample is mixed with 90 ml. of saturated solution of magnesium sulphate and sufficient extra salt to saturate 10 ml. of water. The liquid is kept at about 30° for half-an-hour, then filtered and washed with saturated magnesium sulphate solution. The nitrogen content in the precipitate is determined by Kjeldahl's method, that due to casein previously found, subtracted and the difference calculated to globulin. $N \times 6.48 = \text{globulin}$.

Lactalbumin. Five g. of the sample is diluted with water to 50 ml. and trichloroacetic acid added to give a final concentration of 4%. The mixture is heated to 70° for half-an-hour, allowed to cool, filtered, the precipitate washed with 1% trichloroacetic acid and the nitrogen determined by Kjeldahl's method. The nitrogen due to casein and globulin previously found is subtracted and the difference calculated to albumin. $N \times 6.38 = \text{albumin}$.

Other Nitrogenous Constituents. About 6% of the total nitrogen found in milk is of non-protein nature. The amount tends to rise in samples low in solids-not-fat. Proteose, peptone, amino-acids and ammonia have been identified as well as urea, uric acid, creatine and creatinine.

Separation of the Nitrogenous Constituents of Milk.—G. M. Moir, *Analyst*, 1931, 73, 147 and 228.

Mineral Constituents.

Potassium, calcium, magnesium and sodium, together with traces of iron are present combined as phosphates, citrates and chlorides, together with small percentages of carbonates and sulphates. Iron is present in too small an amount for human requirements.

Colostrum. The milk from mammals shortly after birth of their young differs from normal milk in containing a very high percentage of an albumin closely resembling blood albumin. The proteins it contains are soluble. Colostrum provides readily absorbable nutriment, as the infant's stomach contains no gastric juice at the commencement. It is highly laxative in properties.

The following analyses by Engling show the composition of colostrum from a cow 8 years old:—

| Time after Calving | Specific Gravity | Fat | Casein | Albumin | Sugar | Ash | Total Solids |
|--------------------|------------------|------|--------|---------|-------|------|--------------|
| Immediately | 1.068 | 3.54 | 2.65 | 16.56 | 3.00 | 1.18 | 26.93 |
| After 10 hours | 1.046 | 4.66 | 4.28 | 9.32 | 1.42 | 1.55 | 21.23 |
| " 24 " | 1.043 | 4.75 | 4.50 | 6.25 | 2.85 | 1.02 | 19.37 |
| " 48 " | 1.042 | 4.21 | 3.25 | 2.31 | 3.46 | 0.96 | 14.19 |
| " 72 " | 1.035 | 4.08 | 3.33 | 1.03 | 4.10 | 0.82 | 13.36 |

The average of 22 analyses by Engling of colostrum from different cows showed total solids 28.31, fat 3.37, casein 4.83, albumin 15.85, sugar 2.48, ash 1.78.—Leach, "Food Inspection and Analysis." See also *Analyst*, 1913, 107, for the results of analysis of colostrum from 20 different cows, in which the fat ranged from 1.3 to 9.0%.

Vitamins. Milk contains the vitamins A, D, B₁, C and E (see page 768). Most interest attaches to vitamin C, especially in connection with the pasteurisation of milk (see page 786).

Ascorbic Acid in Milk. Milk as secreted by the cow contains all its vitamin C as ascorbic acid. On exposure to light the ascorbic acid becomes reversibly oxidised to dehydroascorbic acid which is also biologically active. Further exposure results in irreversible changes and loss of activity. A pint bottle of milk when exposed to daylight for 30 minutes and then placed in the dark for 1 hour loses half its content of vitamin C. Pasteurisation by the holder method destroys the dehydroascorbic acid form of the vitamin but not the reduced form.—S. K. Kon and M. B. Watson, *Biochem. J.*, 1936, 2273.

The action of sunlight produces two effects on milk. (1) Oxidation of unsaturated fat. This phenomenon is independent of the decolorisation of methylene blue. The reduction of methylene blue is, however, aided by this oxidation of the unsaturated fat, which produces anaerobic conditions in the milk by using up the dissolved oxygen and thus allows the second phenomenon to appear. (2) Oxidation by catalytic dehydrogenation of the ascorbic acid present in the milk. This dehydrogenation is responsible for the decolorisation of the methylene blue, which serves as hydrogen acceptor. When all the ascorbic acid has been oxidised the colour of the methylene blue is restored if air or oxygen be admitted. The determination of the substances oxidisable by iodine before and after exposure to sunlight can be used to evaluate the vitamin C content of milk. The results obtained by this method agree well with those obtained by direct titration with 2 : 6-dichlorophenolindophenol.—L. Buruiana, *Biochem. J.*, 1937, 1452.

The Freezing-point of Milk. Milk freezes at a lower temperature than water owing to the presence of lactose and mineral salts in solution. The freezing-point is very near -0.545° . The depression of the freezing-point is used in cryoscopic work; thus the average depression of the freezing-point of milk is 0.545° . This is dependent only on the number of molecules and ions in solution, not on the composition of the solids, and is unaffected by the conditions affecting the composition of the milk. The figure for solids-not-fat in milk from single cows or small herds frequently falls below 8.5%, and there is no method other than the freezing-point test which will distinguish with certainty between genuine samples low in non-fatty-solids and those adulterated by addition of water. Moreover the test will detect the addition of water to milk originally high in solids-not-fat, though the analysis shows the figure to be well above 8.5%.

In a Report to the Local Government Board in 1914 Dr. Monier-Williams reviewed the work done in previous years, and gave the results of many experiments carried out by him in an apparatus which he had devised. He obtained an average freezing-point of -0.5345° , the values found ranging from -0.558° to -0.514° .

He concludes that the freezing-point appears to be the most constant of any of the properties exhibited by genuine milk. It is unaffected by the removal of fat, or by the addition of separated milk, but it is raised by the addition of water.

"The method may, in certain circumstances, be applied with advantage, as a confirmatory test, to the detection of added water, and to the approximate estimation of the amount present.

"Owing, however, to the experimental difficulties involved in obtaining reliable results, it is somewhat doubtful whether the method is capable of general application for purposes of milk control."

Dr. Monier-Williams' experiments were carried out under conditions ensuring scientific accuracy, and the values quoted in his report had been subjected to all necessary corrections.

Hortvet Apparatus. In 1917 Hortvet with collaborators working for the *A.O.A.C.* designed an apparatus simple in construction, capable of giving identical results on repeat samples of the same milk, and of giving the same results in the hands of different workers. Cooling is produced by the evaporation of ether as in the Monier-Williams apparatus, the process being more readily controlled than when mixtures of ice and salt are employed. Determinations are made under standard conditions, and no corrections are made for sources of error. The depressions found are probably a little too high, but are strictly comparable with one another. (Elsdon, in *Recent Advances in Analytical Chemistry*, Vol. I, p. 252.)

Several workers have not appreciated that the errors of the method are more or less fixed and largely cancel out, and many results found in literature are based on technique differing from that laid down by the *A.O.A.C.*

The Council of the Society of Public Analysts (*Analyst*, 1933, 58) recommended:

(1) that for administrative purposes the freezing-point of samples of milk should be determined in accordance with the Hortvet technique exactly as described in the *Official and Tentative Methods of Analysis of the A.O.A.C.*, 3rd Edn., 1930; no correction other than those directed therein should be applied:

(2) that the freezing-points thus obtained should be recorded, for example, as

Freezing-point (Hortvet) -0.550° .

The test is used officially in the United States, New Zealand, Germany, Holland and some other countries.

The range of freezing-point depression (Hortvet) lies between 0.525° and 0.565° , the average figure being 0.544° . Elsdon and Stubbs (*Analyst*, 1930, 423) make the following suggestions: "that an average of 0.54° may be taken for the purpose of calculating added water, but that no milk should be considered watered on the evidence of the freezing-point of a single sample alone, unless the depression falls below 0.53° ."

As milk becomes sour the depression of the freezing-point increases so that the method is only applicable to fresh milk or to slightly sour milk (limit of acidity 25°) after correction.

Added Water. A table is given in the *A.O.A.C. Methods of Analysis* for the percentage of added water corresponding to the determined freezing-point depression.

The percentage of added water (W) may also be calculated as follows:—

$$W = \frac{100(T - T')}{T} \text{ where}$$

T = the average freezing-point of normal milk (-0.550°), and T' = the observed freezing-point of a given sample.

Elsdon and Stubbs suggest that where the percentage of added water is small it may be calculated more accurately from the formula:—

$$W = \frac{T - T'}{T} (100 - \text{Total Solids}).$$

The formula gives the percentage by weight.

If the freezing-point lies between -0.550° and -0.530° , the milk may contain added water, in the latter case up to 3% though the figures are normal to genuine milk. Stubbs and Elsdon (*Analyst*, 1934, 146) reported on the Hortvet Test on 1000 milks believed to be genuine. The extreme figures were -0.529° and -0.563° with an average of -0.544° . In U.S.A. no return is made on a F.P. below -0.530° but above that figure the calculation is made from the formula given above. No definite standard exists in Britain; it is suggested that T in the formula be taken as -0.530° for calculation. Though the percentage of added water would usually be understated, there would be no chance of an offence being charged which had not been committed. The act of adding water, not the amount of water added, constitutes the offence.

In the ordinary analysis of milk the amount of water added is obtained from the solids-not-fat figure by an exactly similar formula.

$$W = \frac{100(S - S')}{S} \text{ in which}$$

$S = 8.5$ (the standard laid down by the Board of Agriculture)

S' = the solids-not-fat determined in the given sample.

As 8.5 is an arbitrary minimum figure the amount of added water so calculated will not as a rule agree closely with that given by the freezing-point formula. It is only correct if the original milk contained 8.5% of solids-not-fat.

Test for Nitrates in Milk. Nitrates have never been detected in milk and the presence of nitrates in a sample is good evidence of the addition of water, though all samples of potable water do not contain nitrates.

Preparation of Reagent. Dissolve 0.085 g. of diphenyl-benzidine in a mixture of 50 ml. of water and 450 ml. of nitrogen-free sulphuric acid.

To 5 ml. of milk add 6 drops of a solution containing 20% of mercuric chloride, 5% of ammonium chloride and 20% of concentrated sulphuric acid. Shake for 2 minutes to precipitate proteins, filter the serum into a test-tube containing 2 ml. of the diphenyl-benzidine reagent so as to form a separate layer. In the presence of nitrate a blue colour is formed at the junction of the liquids. The test will detect 1 part of nitrate in 10 million parts of milk.

MILK (SPECIAL DESIGNATIONS) ORDER, 1936. S.R. & O., 1936, No. 356.

3. The special designations which may be used in relation to milk are "Tuberculin Tested," "Accredited" and "Pasteurised."

6. Tuberculin Tested Milk. The producer must satisfy the Licensing Authority that the herd is an "Attested" one or that every animal has passed the prescribed Tuberculin Test within one month of the date of application, and must furnish the Authority with a vet.'s certificate showing the results of an examination carried out within one month of the date of application. Any animal reacting to the Tuberculin Test or showing evidence of disease likely to affect the milk injuriously must be removed from the herd. Accredited Milk. The producer must furnish the Authority with a vet.'s certificate as above.

THIRD SCHEDULE.

Part I. Conditions applying to Tuberculin Tested Milk.

Producers to have every animal examined by a vet. and submitted to a T.T. test every 6 months. No animal which has not passed the T.T. test to be added to the herd. Animals reacting to be removed from the herd. Complete register of animals to be kept. The herd to be isolated from all other cattle. Containers of milk for delivery to customers must be tightly closed, suitably capped and labelled "Tuberculin Tested Milk." Milk shall not be heated at any stage unless a licence has been granted for the sale of such milk as "Pasteurised." T.T. milk shall pass a prescribed methylene blue reduction test and shall contain no coliform bacillus in 0.01 ml. If pasteurised, not more than 30,000 bacteria per ml. is permitted.

Part II. Conditions applying to Accredited Milk.

Producers to have every animal examined by a vet. every 3 months. Unsatisfactory animals to be removed or segregated. Complete register of animals to be kept. Containers of milk for delivery to customers must be tightly closed, suitably capped and labelled "Accredited Milk." Milk shall not be heated at any stage. Bacteriological requirements as for T.T. milk.

Part III. Conditions applying to Pasteurised Milk.

Milk must be retained between 145° and 150°F. for at least half an hour, cooled immediately to 58°F.; must not be re-heated (*Amended 1941, v. infra*). Pasteurised Milk may not contain more than 100,000 bacteria per ml. T.T. milk bottled at the farm may be described as "Tuberculin Tested Milk (Certified)."

Milk (Special Designations Amendment) Order, 1938. S.R. & O., 1938, No. 218.

This order consists of certain minor amendments to the 1936 Order and the two Orders may be cited together as the Milk (Special Designations) Orders, 1936 and 1938.

Milk (Special Designations) Regulations, 1941. P.R. & O., 1941.

1. These regulations to be read as one with the Milk (Special Designations) Order, 1936, as amended, and may be cited, together with the Milk (Special Designations) Orders, 1936 and 1938, as the Milk (Special Designations) Regulations, 1936 to 1941.

SECOND SCHEDULE.

This amends the requirements for pasteurised milk as set out in Part III of the Third Schedule to the Order of 1936. Pasteurised milk is now milk which has been retained at a temperature of not less than 162°F. for at least 15 seconds and the pasteurising apparatus must be thermostatically controlled and be provided with a device which shall automatically divert the flow of milk which has not been so treated.

Milk (Special Designations) Regulations, 1942. S.R. & O., No. 771.

1. These regulations and the Milk (Special Designations) Regulations, 1936 to 1941, may be cited together as the Milk (Special Designations) Regulations, 1936 to 1942.

2. Animals in attested herds may not be inoculated or vaccinated against tuberculosis or vaccinated with live *Brucella abortus* except with the approval of the Minister of Agriculture and Fisheries and with a vaccine approved by him.

Tuberculin Tests in Cattle with special reference to the Intradermal Test. (Spec. Rep. Ser. Med. Res. Coun., Lond., No. 94, 125).

The *subcutaneous test* is satisfactory under laboratory conditions, but not under farm conditions. The *intradermal test* is superior, while the *ophthalmic test* must be regarded as a subsidiary test. The percentage of error with the intradermal test is small; animals diagnosed as tuberculous by this test have not shown tuberculosis on naked-eye examination *post mortem*, but have been proved tuberculous by microscopic examination and guinea-pig inoculation. The test has the advantages over the subcutaneous test that temperature observations are not required, that the animal need not be kept at rest, that it does not interfere with farm routine, that only three observations are usually necessary, that a smaller quantity of tuberculin is needed, and that the technique is easily acquired. "Old tuberculin" (either bovine or human strain) is used; it must be

of proved high potency and is given undiluted. In combining the ophthalmic test with the intradermal test frequent examinations of the animal's eyes are necessary since the reaction when positive is apparent 24 hours after the second instillation.

Modified Intradermal Test. A further report "The Intradermal Tuberculin Test in Cattle" (J. B. Buxton and A. S. McNalty, *Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 122, 1928) by the Tuberculin Committee of the M.R.C., states that the intradermal test is more trustworthy. It involves two injections of tuberculin—a "sensitising" and a "reacting" dose—on different occasions. Can be readily performed under farm conditions, has little or no effect on milk yield and is free from risk in pregnant cows and young animals. Ophthalmic test unreliable.—*Brit. med. J.*, ii/1928, 808.

The double intradermal tuberculin test consists of two injections of concentrated tuberculin into the skin of the neck with an interval of approximately 48 hours. The test is superior to the single intradermal as formerly practised into the caudal fold and also to the conjunctival method. The reaction after double intradermal injection does not appear to be affected by previous testing with tuberculin either by the intradermal or subcutaneous methods. The test is also considered to be superior to the subcutaneous test and is capable of giving a higher percentage of reacting animals than the subcutaneous method, it being exceptional for a positive reaction to occur in a non-tuberculous animal.—J. Cuillé and P. Chelle, per *Bull. Hyg.*, Nov. 1935, 708.

MILK MARKETING SCHEME (APPROVAL) ORDER, 1933, made under Section 1 (8) of the Agricultural Marketing Act, 1931. (A résumé.)

Part II. The Board and the Regional Committees. Sections 5 to 37. The Board to administer the scheme to be called the "Milk Marketing Board," a body corporate, to consist after 30th June, 1934, of 12 regional members elected by the registered producers in the respective regions, three special members elected by the producers in general meeting, and two persons co-opted by the elected members. **Regional Committees** consisting of a member or members of the Board, together with one or more representatives from each of the counties in the region, to report to the Board on the operation of the Scheme in their respective regions.

Part III. Register of Producers. Sections 38 to 41. The Board to keep a register of producers, every producer being entitled to register therein, producers exempt from registration being those with not more than four milch cows (unless they sell milk by retail), and producers not carrying on the business of selling milk.

Part IV. Polls. Sections 42 to 47. On the coming into force of the scheme a poll of the registered producers to be taken on the question as to whether the scheme is to remain in force. (*Result of Poll declared in August 1935 showed a majority in favour of continuance of the scheme.*)

Part V. Financial Provisions. Sections 48 to 51. A fund to be established, and every registered producer to contribute not more than one shilling for every milch cow in his possession.

Part VI. Principal Powers of the Board. Sections 52 to 74. (54) Producers neither registered nor exempt to be prohibited from selling milk. (55) The Board may regulate sales of milk, i.e., the description of milk which may be sold, and the price at which it may be sold. (56) The Board may buy milk, produce commodities from milk (specified in Second Schedule), sell, grade, advertise and transport milk or milk commodities, encourage, promote or conduct agricultural co-operation among milk producers, or research and education in connection with production and marketing. (57) Shall draw up prescribed contracts for the sale of milk by registered producers, the purchase price being paid by the purchaser to the Board. (61) In the event of the registered producer being unable to find a customer under the prescribed terms of contract, it is the duty of the Board to accept the unsold milk. (62) No registered producer may sell milk by retail without a "retail licence" issued by the Board. (63) The Board shall prepare a register of Accredited Producers who will be entitled to an additional prescribed payment out of the fund ("Guaranteed Quality Premium") per gallon of milk sold.

The Tuberculosis (Attested Herds) Scheme (England and Wales).

While the Milk Board proposals for an Accredited Scheme aim at raising the general standard of cleanliness and purity of the milk supply, the Attested Scheme of the Ministry of Agriculture is directed more specifically to the eradication of tuberculosis from dairy herds. The scheme offers a special inducement to this end to dairy farmers. It carries the improvement of the milk supply a stage further than the Milk Board's Accredited Scheme, and those who qualify for the register of attested herds earn an extra bonus from Government funds in addition to the bonus to be offered under the Accredited Producers' Scheme.

The terms fixed for the Ministry of Agriculture's attested register are more exacting than those of the Ministry of Health's T.T. Milk, and it has been suggested by experienced producers that the provisions of the Ministry of Agriculture's scheme are so drastic as to make it unworkable in practice. They have found, in spite of all precautions, that it is well-nigh impossible to keep a dairy herd absolutely free from reactors over a period of years, and that almost always one or two cows fail at the six-monthly tuberculin tests.

Under the present scheme an owner may make application to the Ministry for an official test, provided that no reactors were found in the herd on the occasion of the last two private tests, if carried out with due regard to the intervals prescribed. The application should be supported with certificates to this effect signed by the veterinary surgeon who carried out the tests on behalf of the owner. A certificate of attestation is valid for one year. It will be renewed annually, if desired by the owner of the herd, after a further official test of all cattle in the herd, provided that no reactors are disclosed and the Ministry is satisfied that the herd and premises continue to be suitable for attestation.

If any reactor is found, the renewal of the certificate of attestation will be suspended, the reactor immediately isolated and disposed of as quickly as possible, and the premises thereafter disinfected. A further official test of the non-reactors is to take place not earlier than 60 days after completion of the disinfection. If this reveals no reactor the certificate of attestation will be renewed for 6 months. If any reactor is found as the result of the re-test it must be removed as before and the premises again disinfected. A second official test will then be carried out after the expiration of 60 days, and if any additional reactor is found the herd will be removed from the register of attested herds and the certificate of attestation will be cancelled.

These official tests will be carried out without charge to the owner, and while his herd is on the register the Ministry of Agriculture will pay him a bonus on all milk sold, from the fund provided by the Milk Act, 1934.

The fundamental principles of successful eradication are stated to be (Advisory Leaflet, No. 223):—

- (i) the tuberculin testing of the whole herd,
- (ii) the separation of reactors from non-reactors, and
- (iii) the thorough disinfection of the premises to be occupied by the non-reactors.

The soundest and quickest method of eradication consists in adequate tuberculin testing and the prompt slaughter of all reactors, but as the percentage of reactors in the majority of the herds of this country is high, unfortunately this method is precluded on grounds of cost. The elimination of reactors by sale is less costly, but animals showing clinical signs of the disease or giving tuberculous milk must be reported and dealt with under the Tuberculosis Order of 1925. In addition to the risks of re-infection of a herd by contact with infected stock, contaminated premises, pastures, or water, there is also the danger of introducing the disease by pigs or poultry. Pigs constitute a very likely source of infection as about 50% of tuberculous infection in these animals is of the bovine type. Manure from reactors is also a potential source of infection.

Advantages of Eradicating Tuberculosis in Cattle.

In a leaflet issued by the Ministry of Agriculture, with the above title, it is pointed out that, under the Tuberculosis Order 1925, 350,550 animals were examined and 20,908 slaughtered in 1933, the total amount of compensation paid being £71,827.

During the years 1926-33 (inclusive) £511,221 was paid as compensation for animals suffering from tuberculosis. The total market value of all the cattle slaughtered was £1,265,105, and the value of those animals had they been healthy would have been very much greater. If to this amount is added the depreciation in the value of cattle affected with forms of tuberculosis not dealt with in the Order it will be seen that the total loss sustained reaches an enormous sum.

This progressive wasting disease leads to reduced milk yield, shorter milking life, increased susceptibility to other diseases and loss in beef-value. In addition, milk from affected cows may contain tubercle bacilli and thus constitute a source of infection to persons, particularly children, consuming it.

Diseased cows cannot long withstand the strain of high milk production.

In addition the average milk yield per cow has been shown to increase as tuberculosis eradication progresses.

The requirements of this scheme are exacting and it seems likely that only self-supporting herds of home-bred stock may find it possible to comply with these requirements. It remains to be seen how far the large proportion of dairy farmers in this country who do little or no breeding or rearing of young stock, but rely on cattle markets for the recruitment of their herds, will be prepared to alter their systems of management in order to achieve the ideal aimed at by this scheme. It has been estimated that 30 or 40% of our milk producers are retail purveyors, many of whose farms are situated in or on the fringe of urban consuming areas, where land values are assessed at urban figures. Nearly all these cowkeepers manage their herds on the "floating" principle rather than on a self-supporting basis, since rentals and other costs are considered to be too high to justify the use of any of the land for stock rearing.—*Rep. med. Offr. Minist. Hlth, Lond.*, 1934, 110.

Discussion on the control of tuberculosis in cattle.—*Proc. R. soc. Med.*, 1942, 35, 469.

PASTEURISATION

From the public health point of view the term "Pasteurisation" should be confined to the process of heating milk to *not less than 162°F. for at least 15 seconds*, and experience shows that when properly carried out this is sufficient to destroy virtually any pathogenic organisms without producing appreciable change in the physical and chemical characters of the milk. *M. tuberculosis*, *C. diphtheriæ*, *B. dysentericus*, *B. typhosus* and other organisms of the *typhoid-paratyphoid* group, together with the virus of foot-and-mouth disease, are all destroyed by pasteurisation. Under certain conditions a small proportion of

tubercle bacilli may escape actual destruction, but their virulence is so impaired as to make them harmless. Some strains of streptococci may survive, but not those believed to be responsible for septic sore throats. Pasteurisation therefore affords a simple means of rendering milk reasonably safe as regards risk of transmitting disease. Pasteurisation does not destroy all the non-pathogenic organisms, though their number may be reduced by 99%. The sporing organisms and a small proportion of lactic acid bacteria survive. The great reduction in lactic acid bacteria, enabling milk to keep fresh longer, is the chief advantage which pasteurisation offers from the commercial point of view. Generally speaking, the number of bacteria in milk after pasteurisation is greater in milk which contained a large number of bacteria before pasteurisation than in milk which originally contained relatively few bacteria—this is of practical importance, the bacterial content of pasteurised milk giving an indication of the bacterial content before pasteurisation.

The ideal milk supply would of course be milk obtained from perfectly healthy cows under the cleanest conditions, consumed immediately with the least possible manipulation, but in a highly urbanised country such as England this is impossible, and without some process for preserving the keeping quality of milk a proportion of the population would be forced to curtail or even do without a very important food. By subjecting the average milk of this country to pasteurisation, the destruction of any pathogenic organisms is virtually assured and its keeping qualities are improved.

A committee appointed by the Health Organisation of the League of Nations to investigate various aspects of the milk problem in Europe came to the conclusion that "no raw milk can ever be regarded as completely safe for human consumption" and gave it as their "considered and emphatic opinion that all liquid milk for human consumption should be adequately pasteurised or boiled." (See *Bull. Hlth Org. L. o. N.*, 1937, 496.)

Experimental evidence does not bear out the assertion that bacteria grow faster in pasteurised than in raw milk—the rate of bacterial increase is approximately the same. It is alleged that pasteurisation will encourage lack of cleanliness in milk production, but this is not so, as milk so stale as to be unfit for sale will not have its flavour improved by pasteurisation—moreover, only a milk sufficiently clean before pasteurisation would comply with the requirements of a bacterial count after pasteurisation.

The nutritive qualities of milk do not appear to suffer appreciable change from pasteurisation, except in respect of antiscorbutic property, which can be corrected by orange juice. It is held by some that changes referred to in the physical and chemical characters are indicative of possible subtle alterations in nutritive value, not easily detected or estimated, which are perhaps of far-reaching importance. Even assuming, however,

some depreciation of these hypothetical nutritive values, it is not unreasonable to assume that the impairment would be of the same order as in those characteristics capable of observation, i.e., partial and slight impairment rather than complete destruction, and from a practical standpoint the positive advantages of pasteurisation outweigh any possible slight depreciation of nutritive elements, the existence of which is hypothetical.—Notes on the Pasteurisation of Milk, by J. M. Hamill, *Rep. publ. Hlth med. Subj., Lond.*, No. 17, 1923.

Experiments on rats show that the nutritional availability of calcium and phosphorus in milk is not affected by pasteurisation, and the biological value and true digestibility of the nitrogen are not affected by the holder process. Neither vitamin A itself, nor the pro-vitamin, carotene, is affected, but vitamin B (complex) suffers some loss, probably mainly in the B₁ fraction. Loss also occurs in the vitamin C content, amounting to about 20% of that originally present, but the loss is due to the destruction of the more labile form which is produced by reversible oxidation under the action of light; reduced ascorbic acid, the form in which the vitamin is secreted by the cow, is not affected. Report to the Milk Nutrition Committee (Part I). From the National Institute for Research in Dairying and the Rowett Research Institute.—*Per Analyst*, 1937, 463.

There are no human experiments which demonstrate that pasteurised milk is less nutritive to the young child than raw milk, while there is a vast mass of clinical experience to show that heated milk has been consumed for years by infants and young children without any detectable deterioration in the nutritive condition.—W. G. Savage, *Lancet*, i/1933, 429, 485.

Children of the same initial height and weight within fixed limits, showed that (1) extra milk (raw or pasteurised) generally increased the gain in height over controls to an equal extent, except that on pasteurised milk older girls gained more than younger girls; (2) extra milk increased gain in weight, more in girls than in boys, more in older girls than in younger, and the difference associated with age was greater with raw milk than with pasteurised; (3) *on the selected material there was no evidence of the superiority of either raw or pasteurised milk in increasing growth rate.*—E. M. Elderton, *Ann. Eugen., Camb.*, 1933, 5, 326.

Under purely practical conditions there is no difference in nutritional value between raw and pasteurised milk. Vitamin C is destroyed to the extent of about 20%. This is not due primarily to heat treatment, but to previous exposure to light. Milk as delivered from the cow contains all its vitamin C as ascorbic acid and if pasteurised at once there would be no loss. Exposure to light results in the partial formation of dehydroascorbic acid, which is destroyed by pasteurisation. Infants fed exclusively on milk, whether pasteurised or raw, require supplements of iron and ascorbic acid. Egg-yolk and fruit juice are advised.—*Rep. of Milk Nutrition Cttee*, 1939.

Biological tests show that commercial pasteurisation brings about a definite though small loss in the vitamin B complex and commercial sterilisation destroys some 30% of vitamin B₁, but has no adverse effect on vitamin B₂. Chemical tests show that pasteurisation destroys 10% of vitamin B₁, but is without effect on riboflavin, while the loss of B₁ in sterilised milk is 50%, riboflavin again being unaffected.—*Brit. med. J.*, i/1941, 248.

Relative nutritive value of different forms of milk.—S. K. Kon, *Nature, Lond.*, 1941, 607.

Organisms found in milk. These may be classed as follows:—(i) Acid producing (100 varieties), the principal member of which is *B. acidilactici*; (ii) *B. acidibutyrici* (has very resistant spores, not killed by pasteurisation); (iii) those responsible for fermentation to alcohol, as koumiss, butter milk, red milk, blue milk, etc.; (iv) the mould *Oidium albicans* produces thrush in infants' mouths; (v) *M. tuberculosis*; (vi) *Streptococci* associated with contagious mammitis; (vii) *C. diphtheriæ*; (viii) *B. coli communis* and *B. typhosus*.

Tubercle Bacillus in Milk.

As a result of experiments to determine the thermal death-point of the tubercle bacillus in milk the following conclusions have been drawn:

(1) By using 25 strains of tubercle bacillus no wide difference in the death-point was found.

(2) The thermal death-point is practically similar for human and bovine types.

(3) Previous variations in results due to too little care in carrying out experiments.

(4) 20 minutes exposure at 60°C. required to prevent milk so treated carrying infection to the guinea-pig.

(5) 5 minutes at 70°C. required to ensure the same result.

(6) Of the two combinations of time and temperature factors the former excels the latter when the *food value* of the treated milk is also considered.

(7) Until bovine tuberculosis can be stamped out at its source pasteurisation is the only safe method of rendering milk safe for human consumption.—F. W. Campbell Brown, *Lancet*, ii/1923, 321.

The raising to 190°F. makes milk perfectly safe from contamination with *B. tuberculosis*, and this does not impair its nutritive value.—N. Raw, *Brit. med. J.*, i/1921, 596.

Pasteurisation at 145°F. for 30 minutes ensures a non-infective milk so far as T.B. is concerned.—R. G. White, *Lancet*, i/1926, 222. Pasteurisation in *closed vessels* at that temperature for 30 minutes, then cooling to under 40°F. renders the milk free from tubercle bacilli and all other pathogenic bacteria.—S. G. Moore, *Brit. med. J.*, ii/1926, 855.

While 26 samples of certified milk were free from tubercle bacilli, 17 of the samples contained *Br. abortus* and 16 mastitis streptococci. Of 39 Grade A (T.T.) samples 1 contained tubercle bacilli, 31 *Br. abortus* and 29 mastitis streptococci. Of 43 samples taken from raw milk coming to London in 3000-gallon tanks *every one contained virulent tubercle bacilli* and 27 *Br. abortus*. After pasteurisation not a single one contained either of these organisms in the living state.—F. C. Minett and E. J. Pullinger, *Brit. med. J.*, ii/1933, 1080.

5483 samples of milk coming into and distributed in Aberdeen, Dundee, Edinburgh and Glasgow were examined for tuberculous infection by the Hannah Dairy Research Institute (*Spec. Rep. ser. med. Res. Coun., Lond., No. 189*). Composite churn samples of raw milk from individual farms were infected to the extent of 10%; raw tank-milk samples 37.5%; flash-pasteurised milk 8.2%; holder-pasteurised milk 2.8%; and retailed milk rather over 5%. Of 714 samples of certified and Grade A (T.T.) raw milk only one sample was infected. All the positive samples from holder-pasteurised milk were taken from three types of plants, the remaining five types yielding perfectly satisfactory milk; faulty pasteurisation probably due to improper design of plant or inefficient operative procedures.—*Brit. med. J.*, ii/1933, 1225.

Of 101 samples of milk from 45 tuberculin-tested herds, 1 contained tubercle bacilli and 70 *Br. abortus*. Samples from 63 3000-gallon rail tanks showed all to be contaminated with tubercle bacilli and 53 with *Br. abortus*. Neither organism survived pasteurisation in corresponding samples. *Samples of tank milk had to be diluted from 10 to 1000 times before tuberculous infectivity for guinea-pigs was lost*. Samples of udder milk from cows with tuberculous mastitis could be diluted one million times with clean milk without infectivity for guinea-pigs being destroyed.—E. J. Pullinger, *Lancet*, i/1934, 970.

During 1936 designated milks supplied from eight licensed tuberculin-tested herds were reported to contain tubercle bacilli.—*Rep. med. Offr Minist. Hlth, Lond., 1936, 143*.

All of 102 samples of pasteurised milk collected from a single plant in the course of a year were free from tubercle bacilli, although these organisms were present in 61 of the corresponding raw samples.—E. Humphriss *et al.*, *Lancet*, ii/1937, 152.

Of 116 samples of retailed raw milk, the percentage failing the standard plate-count, presumptive *B. coli*, and methylene blue reductase test were 47, 53 and 47 respectively; during the summer months the corresponding percentages rose to 76, 78 and 66. 20% of the samples contained virulent tubercle bacilli. Compulsory pasteurisation of milk is considered an essential measure.—C. A. Green, *Brit. med. J.*, ii/1942, 10.

Rapid detection of *B. tuberculosis* in milk.

The microscopic test for tubercle bacilli in milk when done on quarter samples (i.e. samples from each quarter of the udder) from individual cows is a very quick and reliable method of diagnosis. Since tubercle bacilli in these films are usually associated with a particular kind of cell-group, it is quicker, easier, and more efficient to examine the films for cell-groups and then to examine these for tubercle bacilli. The tuberculous cell-groups are made up of pale-staining cells larger than other cells in milk and rather loosely piled up. The milk is first centrifuged for 3 minutes at 2500 r.p.m. The tube with the deposit is then held inverted (after pouring off the milk), while a loop bent at a right-angle transfers the deposit to a slide (two loopfuls). This deposit is then spread with another slide (by spreading the deposit with another glass slide rather than with the loop the cell groups tend to accumulate round the edges of the film so that under the microscope the edges can be examined first and if there are no cell groups in this part the slide does not need further attention). The film is then dried in the air for half an hour and fixed in a flame—not too hot. After cooling, place in alcohol and ether equal parts for 15 minutes and then wash with ether to get rid of the fat (if the fat is not removed, time is lost in examining the films because there are many red stained particles which are fat and not tubercle bacilli). The film is then washed in steaming carbolfuchsin for 8 minutes, washed in water, counter-stained with Löffler's methylene blue for 2 minutes and washed well with water before drying in the air. The film is examined under a low-power objective ($\frac{3}{8}$ in.) for the presence of tuberculous cell-groups and only these cell-groups examined with oil-immersion lens for tubercle bacilli. In examining quarter samples when tuberculous cell groups are present, it is almost always possible to find tubercle bacilli in some of them. By this method it is possible to examine 40 films in an hour without difficulty. Quarter samples have been examined by this method from some 950 cows and in only one case has the finding not been the same as the result from the biological examination of the milk or post-mortem examination of the cow.—M. L. Cowan Maitland, *Lancet*, i/1937, 1297.

Certain organisms, particularly those belonging to the so-called lactic group, may fail to stain by the usual methods in milk that has been heated. The direct microscopic count on pasteurised or sterilised milk may consequently be very misleading. Such bacteria can be stained quite satisfactorily, however, if the preparation is (1) exposed to the dye for 10 to 12 hours instead of for only a few minutes, (2) subjected to the Ziehl-Neelsen hot carbolfuchsin process, or (3) treated with 1% acetic acid before staining by the normal methods.—G. Guittouneau and J. Brigando, per *Bull. Hyg.*, 1936, 712.

Milk-borne infections.

During the years 1912 to 1935 records are available in Great Britain of the occurrence of 103 milk-borne outbreaks of scarlet fever, septic sore throat, diphtheria, typhoid fever, paratyphoid fever, dysentery and gastro-enteritis, affecting about 12,000 persons. During the same time, it is estimated that about 150,000 persons contracted tuberculosis of bovine origin, of which over 60,000 died, while an unascertained number, probably several thousands, suffered from undulant fever due to infection with *Br. abortus*.—H. C. Bendixen *et al.*, *Bull. Hlth Org. L.N.*, 1937, 494.

In August 1936 an outbreak of enteric fever in Bournemouth, Poole and Christchurch resulted in 718 notified cases of illness and in 51 deaths among the resident population. The outbreak was caused by the consumption of raw milk supplied by one dealer only; the milk was obtained from 37 producers, one, or possibly two neighbours, of whom supplied infected milk. Cows had access to an infected stream, the infection coming from the overflow of a tank built to take the sewage and storm water of a large house. When the number of occupants was large the tank was unable to deal with the sewage and the overflow was quickly carried to the stream. From time to time a carrier of *B. typhosus*, who was, however, unaware that he had ever suffered from enteric fever, stayed at the house. Thus some 10,000 customers of the milk dealer were potential victims of typhoid fever due to a sequence of events which, apparently a matter of chance, might be repeated anywhere. The modern practice of concentrating the retail distribution of milk (and of other foodstuffs) in fewer hands makes the safeguarding of food a vital necessity.—W. V. Shaw, *Rep. Publ. Hlth med. Subj.*, *London*, No. 81, 1937.

In October 1936 there occurred an outbreak of gastro-enteritis in the Borough of Wilton near Salisbury, in which over 100 schoolchildren were involved.

The infection was traced to a cow which, though apparently healthy, was a persistent intestinal carrier of *Salmonella* of the "Dublin" type. The milk by which the infection was conveyed formed only a part of the total quantity produced by the herd and the remainder was habitually distributed in a pasteurised form and consumed without any ill-effect.—*Rep. med. Offr Minist. Hlth, Lond.*, 1936, 49; see also *Rep. publ. Hlth med. Subj., Lond.*, No. 82, 1938.

In 25 persons, nausea, cramps, vomiting and diarrhoea occurred approximately 24 hours after consuming milk or ice cream (containing milk from the same source). Cultures of the milk showed yellow and white staphylococci. Filtered cultures from the isolated strains of white staphylococci reproduced the symptoms in animals and man.—H. J. Shaughnessy and T. C. Grabb, *J. infect. Dis.*, 1936, 53, 318.

During an outbreak of milk-borne scarlet fever and tonsillitis at Doncaster in December 1936, 364 people were affected. The infecting organism was *Streptococcus pyogenes* Type II.—R. Watson, *Brit. med. J.*, i/1937, 1189.

An outbreak of Sonne dysentery occurred in Bedford in January 1938 due to drinking infected milk, 96 persons being affected and the symptoms lasting from 12 to 36 hours.—G. K. Bowes, *Brit. med. J.*, i/1938, 1092.

Detection of Pasteurisation.

To 5 ml. of the milk add 1 ml. of benzidine acetate solution 1% and a drop of acetic acid; after shaking, 3 ml. of H_2O_2 are run on to the surface of the mixture.

| | | | | | |
|-------------------------|-----|-----|-----|-----|--------------|
| Unheated milk gives ... | ... | ... | ... | ... | Blue colour. |
| Heated milk, about 60° | ... | ... | ... | ... | Faint blue. |
| Heated milk, above 70° | ... | ... | ... | ... | No colour. |

Arnold's Test gives similar reactions, an old tincture of guaiacum being used as reagent, and dropped on to the surface of the milk. Freshly prepared tincture requires the addition of hydrogen peroxide.

Detection of Hypochlorites. In Canada, chemical sterilisation of dairy utensils is legal, and Lockhead has shown the efficiency of chlorine compounds for this purpose. In this country, however, it is illegal because of the difficulty of detecting contamination of the milk by the sterilising chemical. A test, based on the indigo-carmin reaction of Korenman, has been worked out capable of detecting directly 0.5 p.p.m. of sodium chlorate in 1 ml. of milk (and, indirectly, sodium hypochlorite according to the quantity of sodium chlorate) and 7 to 7.5 p.p.m. of available chlorine.—I. V. Hunt, *Analyst*, 1939, 653.

To cooled milk add diluted sulphuric acid containing a trace of stannous chloride. Mix well in an ice-bath. Centrifuge and examine in ultra-violet light for yellow fluorescence. Full-cream milk must be previously diluted.—B. C. Wright and E. B. Anderson, *Analyst*, 1938, 252.

A Bacteriological Test to Detect Pasteurised Food. When food has been pasteurised at 60°, subsequent heating to any temperature less than this does not appreciably reduce the number of bacteria, but heating above 60° would considerably reduce the number. On the other hand, the bacteria in unpasteurised food are reduced progressively as temperature rises from 50° to 60°.—C. E. Dukes, *Brit. med. J.*, ii/1929, 907.

Detection and Determination of Raw Milk in Pasteurised Milk.

30 ml. of sample is heated at 50° for 5 minutes in a 100 ml. flask and quickly cooled. The flask is completely filled with water and connected by rubber tubing about 5 cm. long with the lower end of a Hoyberg butyrometer tube also filled with water. The apparatus is inverted several times to mix the contents (120 ml.) and is then allowed to stand at 12° to 15° for 20 hours. The depth of the cream layer is then read. The factor C is calculated by multiplying the depth of the cream layer by 4 and dividing by the fat content of the undiluted milk. The addition of 5, 10, 15, 20 and 30% of raw milk to a pasteurised milk of fat content 3.0 altered the C value from 3.3 to 8.0, 27.0, 33.3 and 53.0 respectively.—F. Stoppel, *Z. Unters. Lebensm.*, 1937, 73, 327.

The Phosphatase Test. The enzyme phosphatase is always present in raw milk but is destroyed at the temperature necessary for efficient pasteurisation. The absence of phosphatase is an indication that the milk has been adequately heated, while its presence denotes insufficient heating or contamination with raw milk. All the commoner pathogenic organisms including the tubercle

bacillus are killed before the phosphatase is destroyed. When milk containing phosphatase is incubated with disodium-phenyl-phosphate, free phenol is liberated and the quantity obtained, which may be determined by a colorimetric method, is an approximate measure of the phosphatase in the milk.

Reagents. (1) A buffer-substrate solution containing 1.09 g. of disodium-phenyl-phosphate and 11.54 g. of sodium barbitone in a litre of water with a few drops of chloroform as antiseptic. (2) *Folin and Ciocalteu's Reagent.* A solution of 100 g. of sodium tungstate and 25 g. of sodium molybdate in 700 ml. of water is treated with 50 ml. of phosphoric acid (85%) and 100 ml. of hydrochloric acid, and the mixture boiled for 10 hours under a reflux condenser in a 1500 ml. flask with ground-glass connection. After the mixture has cooled, 150 g. of lithium sulphate and 4 to 6 drops of bromine are added and the excess of bromine is expelled by boiling the liquid for 15 minutes; the reagent, which should be golden-yellow in colour without any green tint, is cooled, made up to 1 litre and filtered. One volume is to be diluted with two volumes of water before use. (3) A 14% solution of anhydrous sodium carbonate in water.

When the test is carried out under standard conditions a pale blue colour is produced which in the absence of phosphatase does not exceed a certain value. For the purpose of comparison a 13 ml. tube is used to contain the test solutions and a standard 2.3 Lovibond blue glass is provided for the measurement of the maximum permissible depth of colour.

Short Test (A). To 10 ml. of the buffer-substrate solution contained in a 25 ml. test-tube add 0.5 ml. of the milk and incubate the mixture in a water-bath at 45° to 49° for 10 minutes. Remove the tube and cool to 15° to 20° by immersing in cold water. Add 4.5 ml. of the diluted phenol reagent, allow to stand for three minutes and filter. To 10 ml. of filtrate add 2 ml. of the sodium carbonate solution, mix and place the tube in boiling water for five minutes and again filter. Compare the colour of the filtrate with the standard coloured glass in the Lovibond tintometer. The test is carried out in duplicate, and at the same time duplicate control tests are made thus: Mix 10 ml. of the buffer-substrate solution with 4.5 ml. of the diluted phenol reagent and 0.5 ml. of the milk, allow to stand for three minutes and filter. To 10 ml. of the filtrate add 2 ml. of the sodium carbonate solution, mix and place in boiling water for five minutes and again filter. Compare the colours of the four tubes. **Interpretation:** If only a faint blue colour develops in all four tubes the milk has been heated but not necessarily pasteurised. If the controls show more than a trace of blue colour, i.e. more than about 1.5 Lovibond units in a 13 mm. cell and there is no free phenol in any of the reagents, it is probable that there is a phenol-producing organism in the milk. This does not occur with milk which after pasteurisation has been kept at a satisfactory low temperature. Milk which after pasteurisation has been properly cooled and maintained at a temperature between 55° and 65° for not more than 18 hours should not show in the control tubes more than a trace of blue colour. If with such controls the incubated tubes show a blue colour which is deeper than the standard the milk has not been adequately pasteurised.

While the production of a colour exceeding the standard may be taken as evidence of incomplete pasteurisation or subsequent addition of raw milk, the converse is not invariably true.

Longer Test (B). To 10 ml. of the buffer-substrate solution contained in a 25 ml. stoppered test-tube, add 0.5 ml. of milk and mix thoroughly. Add 2 drops of chloroform, stopper the tube and incubate at 37° to 38° for 24 hours. At the end of this time add 4.5 ml. of the diluted phenol reagent, mix, allow to stand for three minutes and filter. To 10 ml. of the filtrate add 2 ml. of the sodium carbonate solution, mix thoroughly and place in boiling water for five minutes; filter. Compare the colour in a 14 mm. tube with the Lovibond standard 2.3 blue glass in the tintometer. Tests are to be carried out in duplicate and control tests to be made when necessary.

Interpretation. If the colour produced exceeds that of the standard glass the milk has been improperly pasteurised. It must be assumed that either the temperature of pasteurisation has been too low, or the duration of heating has been too short, or a small quantity of raw milk has found its way into the pasteurised bulk.

A table is given showing, i.e., that as little as 0.2% of raw milk added to milk pasteurised at 145°F. can be detected.—Kay and Graham, *J. Dairy Research*, 1935, 6, 191. A critical study of the test.—Anderson, Herschdorfer and Neave, *Analyst*, 1937, 86.

A table is given showing the results of the phosphatase test on designated pasteurised milk sold to the public or to children under the "milk in schools" scheme in London Boroughs and elsewhere. The authors consider that their recent application of the test permits the following statements:—

- (1) "A large number of pasteurising plants of all sizes both in London and elsewhere are either inherently functionally inefficient or unsatisfactorily operated.
- (2) "The properly designed and supervised plants, whether large or small, provide a satisfactory product. The phosphatase test has proved a valuable aid in this supervision.
- (3) "Of the large firms a majority, but by no means all, use efficient plants and/or methods.
- (4) "The milk sold to school-children as 'pasteurised' appears, in a large proportion of the cases examined, to have been pasteurised in a particularly amateurish way.
- (5) "In view of the large percentage of designated 'pasteurised' milks on the market which have been grossly underheated it is particularly desirable that all milk, and especially milk which is to be consumed by school-children, should reach a reasonable hygienic standard—say Grade A—before pasteurisation."—H. D. Kay and F. K. Neave, *Lancet*, i/1935, 1516.

A negative result, i.e. failure to demonstrate the presence of phosphatase, does not necessarily indicate that the milk has been properly pasteurised, since it may not be possible to demonstrate indubitably the presence of phosphatase in milk which has been heated to below the pasteurising temperature, or for rather less than half an hour, or to which a small proportion of raw milk has gained access. Nevertheless, it is claimed that even relatively small errors in the technique of pasteurisation may be detected.—*Rep. med. Offr Minist. Hlth, Lond.*, 1935, 137.

The phosphatase test is a valuable supplement to the inspection of the plant and a positive result affords convincing evidence that pasteurisation has not been efficiently carried out. The obligation to detect in what way the pasteurisation plant or technique is at fault actually rests with the licensee, and if he cannot deliver milk which satisfies the requirements of the phosphatase test he should not continue to hold a licence.—*Rep. med. Offr Minist. Hlth, Lond.*, 1936, 144.

Faulty Pasteurisation.

Though a number of licensed plants are found to be inadequately pasteurising the milk, it is abundantly clear that perfectly safe milk, free from tubercle bacilli, can be, and is being, consistently obtained in many licensed plants by attention to the main factors concerned. Apart from the technical control of pasteurisation, three general proposals are made to improve the present position. (1) That the Milk Marketing Board should offer a relatively larger margin of profit to distributors who agree to take out licences for the sale of "pasteurised milk," thus affording a stimulus to the distributor to instal properly designed and supervised plant. (2) The need for training and certification of plant operators is stressed. (3) An increase is recommended in the facilities available for routine laboratory control of milk. At present this is confined to a few of the larger companies. *An Inquiry into the Design, Operation and Efficiency of Pasteurising Plants*.—A. W. Scott and N. C. Wright, Hannah Dairy Research Institute, 1935, per *Brit. med. J.*, i/1936, 117.

Results which have been obtained show that a stricter control of pasteurisation is very necessary. Tubercle bacilli have in some instances been found in milk sold as pasteurised.—*Rep. med. Offr Minist. Hlth, Lond.*, 1936, 144.

A survey, using the phosphatase test, of the sufficiency of pasteurisation of designated pasteurised milks in London and elsewhere has shown a considerable improvement during the last two years, though in distributing firms of intermediate or small size the efficiency is still far from satisfactory.—W. A. Hoy and F. K. Neave, *Lancet*, ii/1937, 595.

The future of the dairy industry lies in the gradual diminution of the small and often inefficient distributor and its concentration into the hands of large, well-organised firms which are making use of every aid that science can offer to improve the technical efficiency of their business and the high quality of their products.—H. C. Bendixen *et al.*, *Bull. Hlth Org. L.o.N.*, 1937, 500.

The Supervision of Milk Pasteurising Plants, by Sir W. Dalrymple-Champneys.—*Rep. publ. Hlth med. Subj.*, No. 77 1935.

BACTERIOLOGICAL TESTS FOR GRADED MILK

Memo. 139/Foods (Ministry of Health, 1937)

Standards. The following bacteriological standards for the various classes of graded milk are prescribed by the Milk (Special Designations) Order, 1936:—

| | |
|--|--|
| <i>Tuberculin Tested Milk and Accredited Milk.</i> | Tested by a prescribed method must not decolorise methylene blue within $4\frac{1}{2}$ hours if sample is taken between May 1st and October 31st; or within $5\frac{1}{2}$ hours if taken from November 1st to April 30th. Must not contain coliform bacillus in 0.01 ml. |
| <i>Tuberculin Tested Milk (Pasteurised).</i> | Must not contain more than 30,000 bacteria per ml. |
| <i>Pasteurised Milk.</i> | Must not contain more than 100,000 bacteria per ml. |

"It is not necessary that every sample should be submitted to both tests, and licensing authorities which require to have frequent tests made of samples of producer's milk may find it convenient to have most of the samples examined by the methylene blue test alone, reserving the coliform test for occasional use."

Sampling. Paragraphs 2 and 3 give instructions for the collection of samples; paragraph 4 deals with sampling from churns; and paragraph 5 with the labelling of samples.

Transport and Storage of Samples. Paragraphs 8 to 10 give directions for Tuberculin Tested and Accredited Milks and paragraph 11 for Pasteurised Milk and Tuberculin Tested Milk (Pasteurised) for the plate count.

Methylene Blue Test. Paragraphs 12 to 26 deal with the apparatus required, the method of mixing the sample, the method of carrying out the test and precautions in regard to the test. It is emphasised that the reliability of the results of the test depends upon strict observance of the directions given. Specially prepared methylene blue tablets are required; traces of impurities normally present in commercial samples seriously affect the reliability of the test. Test-tubes and pipettes conforming to descriptions supplied are to be used. The milk is to be regarded as decolorised when the whole of the column of milk is completely decolorised, or is completely decolorised up to within 5 mm. of the surface.

Coliform Test for Tuberculin Tested and Accredited Milks. Paragraphs 28 to 34 give the apparatus, medium and diluent required and details of the technique. The sample passes the test if two out of three tubes are found to be free from acid plus gas after 48 hours' incubation at 37°C.

Plate Count Test for Tuberculin Tested Milk (Pasteurised) and for Pasteurised Milk. Paragraphs 35 to 45 give the apparatus, medium and diluent required and details of the technique.

An appendix gives forms on which the results of the tests are to be recorded.

Microscopic Plate Method. To economise material and save time Frost has introduced a modification of the agar count, in which little plates of agar are made on microscopic slides. Areas of 4 sq. cms. are mapped out on slides, sterilised by flaming, 1 ml. of milk to be tested added to an equal quantity of melted agar at 44° and, after thoroughly mixing, 0.1 ml. of the mixture spread out quickly and evenly over the prepared area of the warm slide, i.e., such a plate is prepared from 0.05 ml. of the milk. When the agar is set it is incubated in a moist chamber at 37°. After 4 to 6 hours the plates are dried without cracking the films, fixed in the flame, immersed in a 10% solution of acetic acid in 90% alcohol, stained with Löffler's methylene blue (1 in 4) for 3 minutes, decolorised until the background is a pale blue, and dried without washing; colonies appear dark blue against a pale blue background. Examine with low-power objective; at least 20 fields should be combed. The number of bacteria in 0.1 ml. agar-milk mixture from which the plates were poured may be found by multiplying the number of colonies in a microscopic field by the number of times the area of this field is contained in 4 sq. cms., the area of the little plate; this figure must then be multiplied by the diluting figure to give the number of bacteria per ml. A fairly close approximation is said to exist between the result obtained by Frost's little plate and the standard plate method.

A critical study of the bacteriological technique used in the grading of milk: the plate count, the coliform count, the methylene blue test and miscellaneous tests. Also a critical study of the interpretation of these tests by comparison with hygienic or unhygienic methods of production, together with a discussion of the general principles of bacteriological grading of milk and general conclusions and recommendations.—*Bacteriological Grading of Milk, Spec. Rep. Ser. med. Res. Coun., Lond., No. 206, 1936.*

Until a more standard practice is adopted by bacteriologists (e.g., a faithful carrying out of the technique for examination of graded milks as set out in Memo. 139/Foods by the Ministry of Health) little value can be placed upon reports received, and both time and money are being wasted in submitting milk samples for examination. A quart bottle of pasteurised milk was shaken and divided into 6 parts in sterile bottles, two of which were submitted to each of three laboratories. One report gave a bacterial count of 9270 per ml. and another 3,400,000 per ml. for the same milk, and the results obtained from one laboratory were 147,000 and 3,400,000. To withdraw a licence upon such unreliable data would be most unjust. It is possible that the milk in some cases is insufficiently shaken before examination; more uniform results would be obtained by the use of a mechanical shaker working at a uniform speed.—J. B. Howell, *Brit. med. J.*, ii/1934, 883.

The methylene blue reductase test is much simpler to apply than the plate count and it yields 75% of comparable results. On the whole it is a more stringent test in warm weather and less stringent in cold weather—the opposite of what is needed. The arguments in favour of its adoption as an economy are weak if the cost of the collection is taken into account. If the coliform test has to be performed in addition, the advantage of the reductase test is not so great as would appear.—J. S. Faulds, *Lancet*, i/1937, 949.

Milk for consumption should not be graded. The best milk available should be directed to the human market, and only this milk should be offered to the public. Grading of producer's milk is highly important; grading of milk for consumption has no particular value and merely confuses the public.—H. C. Bendixen *et al.*, *Bull. Hlth Org. L.o.N.*, 1937, 498.

Test for Stale, Sour or otherwise Bad Milk

It is known that the addition of hydrogen peroxide to fresh, pure, clean milk produces only slight evolution of oxygen, while in the case of stale, sour milk, or milk containing pus or blood or from animals suffering from inflamed udders, fevers, etc., the test produces a much larger quantity of oxygen and that more rapidly.

Experiments showed the following results:—

(1) With new milk no gas evolution in the first $\frac{1}{2}$ hour. During the next 2 hours about 0.5 ml. evolved.

(2) With sour milk (about 2 days sour) gas evolved at once. After 5 minutes 1 ml. of gas, after 30 minutes 5 ml. In the next two hours a further 1 ml. (The results were obtained using 50 ml. of milk in a Doremus tube.)

Test for Freshness of Milk (Schmidt-Muller). The reagent, which should be freshly boiled each day, consists of 5 ml. saturated alcoholic solution of methylene blue (zinc chloride double salt) with 195 ml. of distilled water. One ml. of the reagent is mixed with 20 ml. of milk, the surface sealed with paraffin, and the test-tube then kept at 45° to 50°. Fresh milk should remain blue for 12 hours or more, reduction of the methylene blue, in the absence of formalin, being due to bacterial contamination. If the solution is decolorised within 1 hour, the organisms certainly exceed 500,000 per ml.—Kenwood.

Dirt in Milk.

Allowable limit of dirt in milk: Recommendation. Any proportion of dung in milk, however small its amount, is objectionable. Clean milk examined by the recommended method contains less than one part per 100,000 by volume of moist dirt; a limit of two parts is the maximum which can be conceded, though such milk is not necessarily clean milk. For official purposes, having regard to the variation in the experimental results obtained, it is not desirable to recommend legal action in the case of an isolated sample of dirty milk unless the amount of moist sediment, determined by the recommended method, exceeds three parts per volume in 100,000 parts of the sample.

The method adopted for recommendation involves sedimentation and subsequent centrifuging of the deposit. The apparatus consists of a standard glass sedimentation vessel of about 650 ml. capacity gradually tapering at its lower end, to which is attached one of the centrifuge tubes. A long stout glass rod carrying a rubber stopper at one end is required for stirring purposes and for closing the main vessel at its lower end while the centrifuge tube is disconnected. Three standard centrifuge tubes are available having the capacity of the graduated portion respectively 0.020 ml., 0.050 ml. and 0.200 ml. The standard centrifuge is capable of a speed of at least 2000 revolutions per minute, and is of such a size that the measurement across the machine when in action from outer tip to outer tip of the tubes is 10½ inches.

A volume of 200 ml. is convenient for the test. A small quantity of preservative is added and thoroughly mixed; e.g., 0.2 ml. of 40% formaldehyde in 10 ml. of water for each 100 ml. of sample. Sufficient of the sample to fill completely the constricted part of the centrifuge tube chosen is first taken. The tube is then connected to the sedimentation vessel, the apparatus fixed vertically and the remainder of the well-mixed milk poured into the vessel. Finally the original receptacle is washed once or twice with water and the washings added to the milk. A small quantity of ether or petroleum spirit may be added to form a layer during the time of sedimentation which should be at least 72 hours; the solvent prevents the holding up of dirt in any separated fatty matter. The milk should be stirred at intervals of 4 hours during the day; that portion of the dirt which at first is carried up with the cream gradually falls. After the sedimentation is complete the glass rod is lowered into the milk, until the rubber stopper reaches the narrowest part of the sedimentation vessel. The centrifuge tube is then detached. In order that no dirt may be lost any overflowing milk is caught in a small beaker and transferred to the centrifuge tube to be used for the second period of sedimentation. The bulk of the milk from the first centrifuge tube is poured into the second tube the constricted part of which is filled with milk, and the tube attached to the sedimentation vessel. The glass rod is withdrawn and the sedimentation allowed to continue, the milk being intermittently stirred as before, for at least 48 hours. The centrifuge tube is then spun for 2 minutes or longer at 2000 revolutions per minute. The dirt is now wholly collected in the graduated portion of the tube. The milk is poured off as far as practicable, the sediment stirred with distilled water added until the tube is almost full, and the tube again spun. The liquid is drawn off and the washing repeated twice with water thus removing all milk solids. A portion of the sediment is next taken from the centrifuge tube, examined under the microscope and carefully returned to the tube. The sediment is stirred at intervals of 10 minutes during 30 minutes with 2 ml. of normal ammonia solution, the liquid run off and the sediment washed three times with water as before. The sediment is similarly treated with normal hydrochloric acid and water, and the treatment with ammonia, water, hydrochloric acid and water is repeated. The sediment is washed free from chlorides and after spinning to get a flat surface the volume is read off. Several readings should be taken, stirring and centrifuging between each reading. If the second period of sedimentation yields any deposit, it must be treated as the first sediment using

the appropriate tube; the two results are added. The origin of the dirt may be ascertained by microscopical examination of the sediment withdrawn for the purpose.—*Report of Sub-Committee of Society of Public Analysts.*

Cellular Elements present in milk are best stained by Jenner's or May-Grunwald's stain. Sodium chloride of either 0.7%, 0.8% or 0.9% not suitable for washing the cells deposited by centrifuge. Washing with ox serum gave better results, causing the least contraction of the cells of any of the wash liquors tried.—Prof. Hewlett, *Lancet*, i/1915, 855.

Normal milk contains polynuclear and polymorphonuclear leucocytes, which may be mistaken for pus cells; as many as 54,300,000 per ml. have been observed in an apparently normal sample. Mere cell counts do not afford a true criterion of pathological condition of the udder; on the other hand a paucity of cells might also indicate a pathological process.

All milk contains leucocytes, but do these become converted into pus cells, and how distinguish one from the other? The cell count is increased in milk taken from a cow which is drying off, but the condition is entirely physiological, not pathological. Differential staining should be done by Jenner's method. If an abscess is deep, and has infiltrated the gland, its presence is shown by increased number of phagocytic cells; if acute, the phagocytic activity of the numerous cells is marked; if chronic, and beginning to be shut off by fibrous tissue, the polymorphonuclear cells are less numerous and less sharply defined. The other cells do not appear to be increased in number.—P. C. Varrier-Jones, *Lancet*, ii/1924, 537.

Counting the Leucocytes in Milk. Exactly 1 ml. of milk is transferred to a special centrifuge tube which is subsequently filled with Toison's Fluid (see p. 673). After mixing, the liquid is centrifuged for 10 minutes. The cream is then broken up and the mixture again centrifuged for an additional 5 minutes. The supernatant liquid is removed down to the 1 ml. mark on tube and the deposit incorporated with the last ml. is stirred round with a wire. The number of leucocytes in this ml. is counted with the ordinary Thoma-Zeiss blood counting chamber.

MILK PRESERVATIVES

See also Food Preservatives, p. 836.

By the *Public Health (Milk and Cream) Regulations 1912*, which apply to the whole of England and Wales, *the use of preservatives in milk is prohibited. "No person shall add, or order or permit any other person to add, any preservative substance to milk intended for sale for human consumption, and no person shall sell or expose or offer for sale, or have in his possession for the purpose of sale, any milk to which any preservative substance has been added."*

Boric Acid in Milk: Detection (1 in 500 will preserve).

This is detected by evaporating and incinerating at least 10 g. of the milk and acidifying the ash with dilute hydrochloric acid (using litmus). A strip of turmeric paper is now placed in the capsule, so as to be only partly wetted by the liquid. Evaporate to dryness at 100°.

If boron compounds are present, the part immersed in the liquid will turn brownish-red (formation of rosocyanin). On moistening with a drop of caustic soda, green and purple colours will be produced. On re-acidifying with hydrochloric acid, the red colour is restored, and is again changed to green and blue with excess of alkali.

Alternatively make the milk or other substance just alkaline with barium hydroxide solution, evaporate and incinerate. Add a few drops of dilute hydrochloric acid, a saturated solution of oxalic acid and an alcoholic curcumin or turmeric solution, dry on the water-bath and take up with a little alcohol. Boric acid or its salts give intense magenta-red. Reaction is different from, and far more delicate than the ordinary turmeric test.—Kenwood.

The flame test is well-known. Evaporate to dryness, treat the ash with a few drops of strong sulphuric acid, add a little methyl alcohol, and apply a light. The alcohol will burn with green at the edges of the flame (at the moment of ignition more particularly). Boric acid 1 in 5000 is shown with ease by this method using 10 ml. of the sample. It will show even 1 in 8000 but with some uncertainty.

Borax and boric acid cannot be differentiated, as borax alone without the use of sulphuric acid gave the colour even though the ash of the milk alone was alkaline to phenolphthalein. If boron is found, titration of the ash would be the only means of concluding in which form it existed by comparing with an average milk residue boron free.

Determination of Boric Acid, Thomson's Method: To 100 ml. of milk add 1 to 2 g. of sodium hydroxide and evaporate the whole to dryness in a platinum dish. Char thoroughly, avoiding a strong red heat; digest the contents of the dish with about 20 ml. of hot water, then add hydrochloric acid carefully drop by drop until the solution is acid. Transfer the contents of the dish to a 100 ml. flask keeping the volume below 60 ml., add 0.5 g. of solid calcium chloride, and a little phenolphthalein, then 10% caustic soda solution until a slight permanent pink colour is produced. Add 25 ml. of lime water, make up to 100 ml. and mix well. Filter through a dry filter paper. To 50 ml. of filtrate add N/1 sulphuric acid until the pink colour disappears; add a drop of methyl orange, and more normal acid until the solution is just neutral to methyl orange. Boil for 3 to 4 minutes. Cool, and carefully make the solution neutral to methyl orange with N/10 alkali. Add sufficient neutral glycerin to ensure that a third of the titrated liquid is glycerin, then add a little more phenolphthalein, and titrate with N/10 sodium hydroxide, each ml. of which is equivalent to 0.0062 g. of boric acid.

For a modification of Thomson's method used in the Government Laboratory, see *Analyst*, 1923, 416.

Formaldehyde in Milk: Detection. A teaspoonful will preserve 10 gallons of milk for 3 days in hot weather.

A large addition can be detected by warming, but it is better to distil the milk; the distillate has the odour of formaldehyde, but the preservative is not wholly volatilised even when evaporated to dryness at 100°. In employing colour tests for formaldehyde a notably weaker reaction is obtained when milk containing formaldehyde is distilled and the distillate tested than when water containing the same proportion of formaldehyde is similarly treated.

Schiff's Reagent. Mix 40 ml. of 0.5% solution of magenta with 250 ml. of water, add 10 ml. of sodium bisulphite solution sp. gr. 1.375, and then 10 ml. of pure strong sulphuric acid; allow to stand for some time, when it will become colourless. It may also be prepared when required for use by adding sufficient of a solution of sulphurous acid to decolorise some of the magenta solution. If the sulphurous acid is added in large excess, traces of formaldehyde will not be indicated. Reddish-violet colour proves presence of formaldehyde. *Other aldehydes, including aromatic aldehydes, also give the reaction.*

It is better to distil as above mentioned or to use Hehner's Test, i.e., purplish-violet ring on layering milk on to strong sulphuric acid; but this is also a group reagent for various aldehyde bodies.

The presence of formaldehyde 1 part in 200,000 can be detected with this test also by the following modification:—

If to the distillate from a sample of milk one drop of a dilute aqueous solution of phenol is added and the mixture poured upon some strong sulphuric acid in a test-tube, a bright crimson ring appears.

Phloroglucin Test. To 5 or 10 ml. of the milk add 5 drops of 1% aqueous phloroglucin solution; shake and add 5 drops of sodium hydroxide solution (30%). Salmon colour (not yellowish tint) indicates addition of formaldehyde. *This test will show 1 of formaldehyde (actual) in 50,000 of milk.*

Rimini's Test. A satisfactory confirmatory test, being almost specific for formaldehyde. For method of applying see *Vol. II* (21st Edn.), p. 554. *This test will show 1 of formaldehyde (actual) in 100,000 of milk.*

Formaldehyde added to foods tends to derange metabolism. Wiley in United States investigated the effects of doses of 100 to 200 milligrammes of formaldehyde (given with milk) on 12 men during 15 days, the total being 2.5 g. to each man. Burning in throat, itching rash, retardation of nitrogen and sulphur metabolism, acceleration of phosphorus metabolism, and loss in bodyweight were observed. Apart from harmfulness as a milk preservative, its use is inadvisable, as in dilute solution it prevents the growth of acid-forming bacteria while not retarding many harmful organisms.

Determination of Formaldehyde. The method of Shrewsbury and Knapp (*Analyst*, 1909, 12) may be used for estimating the amount of formaldehyde in a sample of milk. The following modification has been found to give very satisfactory results.

The reagent (100 ml. of concentrated hydrochloric acid and 0.1 ml. of concentrated nitric acid) becomes yellow very rapidly and masks the colour given by the formaldehyde. To overcome this the acids are added separately to the milk, and the whole then mixed. Add 10 ml. of hydrochloric acid to 5 ml. of the sample contained in a test-tube, then add 1 drop of 5N nitric acid from a pipette, shake and place in a water-bath at 50° for 10 minutes. The tube must not be allowed to touch the bottom of the water-bath. A series of tubes with fresh milk containing added amounts of formaldehyde ranging from less than 1 to, say, 10 parts per million should be treated exactly as above, a blank experiment with the milk used being put on at the same time to ensure its freedom from formaldehyde. After 10 minutes the tubes are removed from the bath, and the intensity of the violet colour in the sample compared with that of the standards.

To prepare standards. Determine the formaldehyde in the formaldehyde solution. Say 40%.

A. Dilute 1 ml. to 100 ml. ∴ 1 ml. of A contains $\frac{0.4}{100} = 0.004$ g.

B. Dilute 1 ml. of A to 100 ml. ∴ 1 ml. of B contains $\frac{0.004}{100} = 0.00004$ g.

Each 0.1 ml. of B made up to 5 ml. with milk represents 0.8 parts of formaldehyde per million.

CONDENSED MILK

The changes in the condition of the milk as a result of condensation are profound and not merely caused by deprivation of water.

In the manufacture of sweetened condensed milk, the maximum temperature reached is usually between 80° and 90°, at which temperature it is kept for a few moments. This is not enough to kill many types of bacteria.

Sweetened condensed milk is never sterile; sporing aerobic bacilli have been isolated from 92% of tins and are probably present in every sample—decomposition does not necessarily follow. The “blowing” of tins of sweetened condensed milk is almost invariably due to growth and chemical activities of yeasts but there is no suggestion that these are harmful.

In *unsweetened* condensed milk, the milk is boiled down under reduced pressure. The sealed tins are heated to 110° or 116° for from 30 to 40 minutes, the tins being rotated to increase penetration. About 80% of samples are found to be sterile, the non-sterile containing chiefly spore-bearing aerobes in small numbers, yeasts being of small significance. Decomposition in condensed milk is nearly always due to non-sporing organisms. Longer processing at lower temperature would give results as good as shorter time at higher temperature, without risk of damage to milk.—“Studies in Sweetened and Unsweetened (Evaporated) Condensed Milk,” Food Investigation Board of Dept. of Sci. and Indust. Res., W. G. Savage and R. F. Hunwicke, *Brit. med. J.*, ii/1923, 296; *Lancet*, ii/1923, 529.

The Public Health (Condensed Milk) Regulations, 1923 and 1927 include the following selected articles and rules.

PART I

Article 2. (1) In these Regulations unless the context otherwise requires:—

“Condensed Milk” means milk or skimmed milk which has been concentrated by the removal of part of its water, whether with or without the addition of sugar, and includes the article commonly known as “evaporated milk,” but does not include the article commonly known as “dried milk” or “milk powder”;

“Skimmed Milk” includes separated or machine-skimmed milk;

Percentages shall be calculated by weight.

PART II

Article 4a. “Where a tin or other receptacle containing condensed skimmed milk is required by Article 4 of these Regulations to be labelled, no person shall expose or offer for sale such a tin or receptacle in a paper or other wrapper unless such wrapper has printed on the outside thereof the words ‘unfit for babies,’ such words being contained within a surrounding line. The type used for the words shall be not less than a quarter of an inch in height, and the printing shall otherwise conform with the rules prescribed for the printing of the same matter on the label affixed to the tin or other receptacle.”

The First Schedule

RULES WITH RESPECT TO THE LABELLING OF CONDENSED MILK

1. Every tin or other receptacle containing condensed milk shall bear a label upon which is printed such one of the following declarations as may be applicable or such other declaration substantially to the like effect as may be allowed by the Minister:—

(i) In the case of full cream milk (unsweetened):—

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| CONDENSED FULL CREAM MILK, UNSWEETENED THIS TIN CONTAINS THE EQUIVALENT OF (a) PINTS OF MILK |
|--|

(ii) In the case of full cream milk (sweetened):—

| |
|--|
| CONDENSED FULL CREAM MILK, SWEETENED THIS TIN CONTAINS THE EQUIVALENT OF (a) PINTS OF MILK, WITH SUGAR ADDED |
|--|

(iii) In the case of skimmed milk (unsweetened):—

CONDENSED MACHINE-SKIMMED MILK (or CONDENSED
SKIMMED MILK), UNSWEETENED

UNFIT FOR BABIES

THIS TIN CONTAINS THE EQUIVALENT OF
(a) PINTS OF SKIMMED MILK

(iv) In the case of skimmed milk (sweetened):—

CONDENSED MACHINE-SKIMMED MILK (or CONDENSED
SKIMMED MILK), SWEETENED

UNFIT FOR BABIES

THIS TIN CONTAINS THE EQUIVALENT OF
(a) PINTS OF SKIMMED MILK, WITH SUGAR ADDED

2. The declaration shall in each case be completed by inserting at (a) the appropriate number in words and figures, e.g., "one and a half (1½)," any fraction being expressed as eighths, quarters or a half.

For the purposes of these Rules milk means milk which contains not less than 12·4% of milk solids (including not less than 3·6% of milk fat) and skimmed milk means milk which contains not less than 9% of milk solids other than milk fat.

The Second Schedule

All condensed milk shall contain not less than the appropriate percentages of milk fat and milk solids as specified in the following Table:—

| Description of Condensed Milk | Percentage of milk fat | Percentage of all milk solids, including fat |
|--------------------------------|------------------------|--|
| 1. Full cream, unsweetened ... | 9·0 | 31·0 |
| 2. Full cream, sweetened ... | 9·0 | 31·0 |
| 3. Skimmed, unsweetened ... | — | 20·0 |
| 4. Skimmed, sweetened ... | — | 26·0 |

In addition to the above grades there is now included (S.R. & O., 1940, No. 1662 and No. 1895) Special Full-Cream Sweetened, containing not less than 10% of butter-fat.

Condensed Milk (Milk Content) Order, 1940. S.R. & O., 1940, No. 1896. Prohibits the sale (except under licence granted by the Ministry) of any full-cream unsweetened condensed milk unless it contains not less than 7·8% by weight of milk fat and not less than 25·5% by weight of all milk solids, including fat.

Standard processes for the examination of Condensed Milk will be found in the *Analyst*, 1927, 402; 1930, 111; and 1932, 630.

The method outlined below has been found to give consistent and satisfactory results.

The determination of the "equivalent pints" of milk as a check upon the statement appearing on the label is an important part of the analysis. The net weight of condensed milk must therefore be known.

Weigh the tin and contents before opening; after opening, transfer the well-mixed contents to a suitable receptacle, wash and dry the empty tin, and weigh.

Prepare a 20% w/v solution by mixing 40 g. of condensed milk with water and diluting to 200 ml.

Total Solids. Evaporate 5 ml. of the solution in a platinum dish, and dry in a water-oven to constant weight. Sweetened condensed milks are liable to give somewhat high results unless the solution is mixed with purified ignited sand before evaporation.

Ash. Evaporate 5 or 10 ml. to dryness and ignite at a low red heat.

Protein. Evaporate 5 or 10 ml. of solution for the determination of nitrogen by the Kjeldahl process, using the factor 6.38 to convert to protein.

Fat. An approximate figure may be obtained by the Gerber process, but repeated whirlings are necessary especially with a 20% solution.

More accurately, treat 10 ml. by the Gottlieb method (see Milk, p. 775) or follow the directions given for this process in the *Analyst*, 1927, 408.

Sugars. Dilute 20 ml. of the 20% solution to 200 ml., to produce a 2% solution.

Lactose. Determine by the volumetric method of Lane and Eynon (*J. Soc. chem. Ind., Lond.*, 1923, 32-37T).

The process consists in the titration of Fehling's solution with the use of methylene blue as an internal indicator. It has the further advantage that the milk or condensed milk dilution need not be treated by clarifying agents to remove protein and fat.

To 10 ml. of Fehling's solution in a conical flask, add 10 ml. or more of sugar solution; boil for 2 minutes and then titrate the boiling liquid with more sugar solution added at fifteen-second intervals until the copper is nearly all reduced; add 3 to 5 drops of a 1% solution of methylene blue, and continue carefully until the colour of the indicator is discharged. The boiling should be continued uninterruptedly throughout the operation.

A second and more accurate titration may now be made as follows. To 10 ml. of Fehling's solution add nearly the whole of the sugar solution (to within about 1 ml.) before commencing to heat. Bring the mixture to the boil, and boil for 2 minutes; add the indicator and complete the titration within one minute.

The authors give tables of factors for various sugars for 10 ml. and 25 ml. of Fehling's solution, these factors varying slightly according to the volume of titrating liquid used. The tables also allow for the effect of the presence of sucrose on invert sugar, etc., and in particular on lactose (*J. Soc. chem. Ind., Lond.*, 1927, 434T) for convenience in analysis of condensed milk products.

It is best, however, to standardise the Fehling's solution with known solutions of lactose, etc., under the conditions of experiment, unless the authors' directions are strictly adhered to.

Sucrose. To 50 ml. of the 2% solution add 1 ml. of a 50% solution of citric acid and heat in a thoroughly boiling water-bath for half an hour. Cool, make up the volume to 100 ml. and titrate 10 ml. of Fehling's solution as before. Express the result as sucrose per cent., using the factor found for the Fehling's (approx. 0.0475 g. per 10 ml.), and deduct from it the equivalent in sucrose of the lactose found before inversion.

The percentage of sucrose in the sample is then deducted from that of the total solids to obtain the Total Milk Solids per cent.

Calculation of Equivalent Pints (*Analyst*, 1923, 597).

All tins bear on the label the number of pints of milk to which the contents are equivalent.

By Rule 2 of the regulations, milk contains not less than 12.4% of milk solids (including not less than 3.6% of milk fat).

The specific gravity of such Full Cream Milk is taken as 1.032.

Let *T.M.S.* be the percentage of total milk solids.

" *S.N.F.* " " " " solids not fat.

" *F* " " " " fat.

" *W* " " " weight of contents of the tin in grammes.

For a full cream condensed milk we have:

$$\text{Total milk solids in tin} = T.M.S. \times \frac{W}{100}$$

1 pint = 20 fl. oz. = 20 × 1.032 = 20.64 oz. by weight.
12.4% of this = 2.559 oz. of total milk solids in 1 pint of milk.
= 2.559 × 28.35 = 72.55 grammes.

$$\therefore \text{Equivalent Pints} = \frac{T.M.S. \times W/100}{72.55} = \frac{T.M.S. \times W}{7255}$$

Similarly, equivalent pints are given by the formulæ:—

$$\frac{F \times W}{2106} \text{ and } \frac{S.N.F. \times W}{5149}$$

In the case of a skimmed milk containing not less than 9% of milk solids other than milk fat (sp. gr. 1035.5) $E.P. = \frac{S.N.F. \times W}{5284}$

Condensed milk should be tested for *poisonous metals*, for boric acid and other preservatives, and for thickening agents such as gelatin and starch.

Stokes' Test may be employed for the detection of *gelatin*: Dissolve mercury in twice its weight of nitric acid, and dilute the resulting solution of mercuric nitrate to 25 times its volume with water. Mix condensed milk diluted with water with an equal volume of this reagent and shake well. Allow to stand for a few minutes, and filter. To the filtrate add a saturated aqueous solution of picric acid in about equal volume. An immediate yellow precipitate is formed if gelatin is present.

DRIED MILK

Abstracts from the Public Health (Dried Milk) Regulations, 1923 and 1927.

"Dried Milk" means milk, partly skimmed milk, or skimmed milk, which has been concentrated to the form of powder or solid by the removal of water;

"Skimmed Milk" includes separated or machine-skimmed milk;

Percentages shall be calculated by weight.

(2) These Regulations apply to dried milk to which no other substance has been added and to the dried milk contained in any powder or solid of which not less than 70% consists of dried milk.

Article 4 lays down the conditions of sale with respect to labelling, and requires the content of milk-fat to be:—

In the case of milk described as dried full cream milk not less than 26%;

In the case of milk described as dried three-quarter cream milk not less than 20%;

In the case of milk described as dried half cream milk not less than 14%; and

In the case of milk described as dried quarter cream milk not less than 8%.

Article 4a. "Where a tin or other receptacle containing dried skimmed milk is required by Article 4 of these Regulations to be labelled, no person shall expose or offer for sale such a tin or receptacle in a paper or other wrapper unless such wrapper has printed on the outside thereof the words 'unfit for babies,' such words being contained within a surrounding line. The type used for the words shall be not less than a quarter of an inch in height and the printing shall otherwise conform with the rules prescribed for the printing of the same matter on the label affixed to the tin or other receptacle."

The Schedule

CHIEF RULES WITH RESPECT TO THE LABELLING OF DRIED MILK

(1) Every tin or other receptacle containing dried milk (other than dried milk to which sugar or some other substance has been added) shall bear a label upon which is printed such one of the following declarations as may be applicable or such other declaration substantially to the like effect as may be allowed by the Minister:—

(i) In the case of full cream milk, that is to say, dried milk containing not less than 26% of milk fat:—

DRIED FULL CREAM MILK
THIS TIN CONTAINS THE EQUIVALENT OF
(a) PINTS OF MILK

(ii) In the case of partly skimmed milk, that is to say, dried milk containing not less than 8% but less than 26% of milk fat:—

DRIED PARTLY SKIMMED MILK
[(b) CREAM]
SHOULD NOT BE USED FOR BABIES EXCEPT
UNDER MEDICAL ADVICE
THIS TIN CONTAINS THE EQUIVALENT OF
(a) PINTS OF (b) CREAM MILK

(iii) In the case of skimmed milk, that is to say, dried milk containing less than 8% of milk fat:—

DRIED MACHINE-SKIMMED MILK
(OR DRIED SKIMMED MILK)

UNFIT FOR BABIES

THIS TIN CONTAINS THE EQUIVALENT OF
(a) PINTS OF SKIMMED MILK

(2) The label on any tin or other receptacle containing *dried milk to which sugar or some other substance has been added* shall be in the appropriate form prescribed in subdivision (1) hereof, with the following modifications:—

(i) There shall be added to the heading the word "Sweetened" if the only substance added to the milk is sugar; the word "Modified" if the only substance added is a constituent of milk, and the word "Compounded" in every other case; and

(ii) The words "with (c) added" shall be added to the last sentence in each case, words being inserted at (c) to specify the substance or substances added.

(3) The declaration shall be completed as follows:—

(i) There shall be inserted at (a) the appropriate number in words and figures, e.g., "one-and-a-half (1½)," any fraction being expressed as eighths, quarters or a half.

(ii) There shall be inserted at (b) the word "Three-quarter" if the percentage of milk fat is not less than 20; "Half" if such percentage is less than 20 but not less than 14; and "Quarter" if such percentage is less than 14 but not less than 8.

(4) For the purposes of this Rule the terms "*Milk*," "*Three-quarter cream milk*," "*Half cream milk*," and "*Quarter cream milk*" mean

milk containing not less than the following percentages of milk fat and milk solids, that is to say:—

| | Milk Fat | Milk Solids (including fat) |
|-----------------------------|----------|--------------------------------|
| Milk | 3.6 | 12.4 |
| Three-quarter cream milk... | 2.7 | 11.6 |
| Half cream milk | 1.8 | 10.8 |
| Quarter cream milk | .9 | 9.9 |

and "*Skimmed milk*" means milk which contains not less than 9% of milk solids other than milk fat.

Formulae for Calculation of Equivalent Pints (*Analyst*, 1924, 471).

W is the total weight of dried milk in grammes, and *F*, *T.M.S.*, and *S.N.F.* are the percentages by weight of fat, total milk solids, and solids-not-fat respectively, in the dried milk.

| | | |
|--------------------------|------------------|---|
| Milk | Equivalent pints | $= \frac{F \times W}{2107}$ or $\frac{T.M.S. \times W}{7258}$ |
| Three-quarter cream milk | " " | $\frac{F \times W}{1582}$ or $\frac{T.M.S. \times W}{6797}$ |
| Half cream milk | " " | $\frac{F \times W}{1056}$ or $\frac{T.M.S. \times W}{6335}$ |
| Quarter cream milk | " " | $\frac{F \times W}{528}$ or $\frac{T.M.S. \times W}{5811}$ |
| Skimmed milk | " " | $\frac{S.N.F. \times W}{5285}$ |

Analysis

Moisture (loss at 100°).

Dry 2 g., in a large platinum dish, to constant weight.

Ash

Incinerate the above at a low red heat in a muffle furnace.

Alkalinity of Soluble Ash

Wash the ash on to a filter and continue washing with hot water until about 200 ml. of filtrate is collected. Titrate the filtrate with N/10 sulphuric acid, using methyl orange as indicator, and calculate the result as sodium carbonate.

Proteins (Kjeldahl).

Take 1 g. of sample, 20 ml. of concentrated sulphuric acid and 10 g. of potassium sulphate, with a small crystal of copper sulphate. Heat gently in a long-necked flask until frothing ceases and continue heating until clear over a moderate bunsen flame. Cool, add water and pour into a large round-bottomed flask (1½ litre); wash the digestion flask with successive quantities of water making up to a total volume of about 500 ml. Make alkaline with strong caustic soda solution (500 g. per litre) and distil into 50 ml. of N/10 sulphuric acid until all the ammonia has passed over. The condenser tube should dip below the surface of the standard acid in the receiver until near the end of the distillation. Titrate the distillate with N/10 alkali using methyl orange or methyl red as indicator. A blank experiment should be carried out and the amount of acid neutralised deducted from that neutralised in the determination.

Each ml. of N/10 sulphuric acid neutralised by the ammonia in the distillate is equivalent to 0.0014 g. of nitrogen.

Use the factor 6.38 to obtain the equivalent of protein.

Lactose

Dissolve 2 g. of sample as completely as possible, make up volume to 200 ml., mix well, and allow to stand.

Use the decanted liquid to titrate 10 ml. of Fehling's solution by the method of Lane and Eynon (see Condensed Milk).

If desired, a clarified solution for titrating may be prepared as follows:

Rub the dried milk into a thin paste with warm water in a mortar and transfer to a 200 ml. flask. Add 0.5 ml. of 50% citric acid solution, then 5 ml. of alumina cream, warm gently on a water-bath, shake well, make up volume, after cooling, to 200 ml., mix and filter.

To prepare alumina cream, which should be fresh, stir ammonia into a saturated solution of alum until the mixture is alkaline to litmus; then add sufficient of the alum solution to make the mixture just acid to litmus.

Fat: by the Werner-Schmidt method (*see* p. 775).

Take 1 g. of dried milk, mix thoroughly with 5 to 8 ml. of warm water, add 10 to 15 ml. of strong hydrochloric acid and proceed in the same way as in the case of milk but make at least 4 or 5 extractions with ether.

If any foreign matter appears in the fat, extract the fat with petroleum ether, weigh the residue, and subtract the weight of this from the original weight.

Samples should also be tested for starch, boric acid, salicylic acid, and poisonous metals.

Methods of Analysis of Dried Milks Adopted by the Milk Products Sub-Committee of the Society of Public Analysts.

Moisture and Total Solids. About 1 g. of sample is placed in a metal (e.g., aluminium) dish, about 2 in. diameter, and 1 in. deep, which has been heated at 102° to 103° for 1 hour and weighed. The sample is dried at 102° to 103° until the loss in weight between successive hourly weighings does not exceed 0.0005 g. and is then cooled and weighed. Loss in weight is moisture and the residue gives the weight of total solids in the sample.

Fat. About 1 g. of sample is transferred to a hard-glass stoppered boiling-tube and 8 ml. of water and 2 drops of strong solution of ammonia are added. The mixture is boiled until lumps are disintegrated, then 10 ml. of hydrochloric acid is added and the mixture heated gently to boiling and maintained at that temperature for 3 minutes. After cooling, 10 ml. of alcohol is added and mixed well and then 25 ml. of ether. The tube is closed with a water-moistened stopper, shaken well for 15 seconds, cooled and the stopper removed and washed, together with the neck of the tube, with light petroleum (b.p. 40° to 60°) more being added to make a total of 25 ml. The re-moistened stopper is replaced, the tube vigorously shaken for 30 seconds and either allowed to stand until two layers form or whirled in a centrifuge. By means of a siphon or wash-bottle fitting (using a cork) transfer the ethereal layer to a flask, wash the tip of the siphon tube with ether (into the flask), wash the interior of the extraction tube with ether, siphon off this ether and wash the tip of the tube as before. Add 15 ml. of ether to the extraction tube, using it to wash the cork and inner limb of the siphon tube before removing it, replace the moistened stopper, shake for 15 seconds, add 15 ml. of light petroleum, washing the neck and stopper as before, shake for 15 seconds, and after separation transfer the ethereal layer to the flask. Repeat the extraction with the same quantities of ether and light petroleum. Distil off the solvents, and dry the residue at 102° to 103° for 1 hour, removing solvent vapour from the flask by a gentle current of air. Cool and weigh, repeating the drying and weighing until no further loss occurs. Completely extract the fat from the flask by washing repeatedly with light petroleum, allowing sediment to settle before decantation, and washing off any fat which may have crept over the edge of the flask during decantation. Dry at 102° to 103° as before. The loss in weight before and after the light petroleum extraction represents fat uncorrected for the blank, which must be ascertained by a determination with the same quantities of reagents.—*Analyst*, 1936, 105.

RECONSTITUTED OR SOPHISTICATED MILK

The establishment of the Milk Marketing Board and the elimination of "cut-price" wholesale milk has led to new devices to enhance the value of skimmed milk. Skimmed milk powder is a cheap article of commerce and if dissolved in water and cream added, the resulting solution is not easily distinguishable from natural cow's milk: sold at a shilling a gallon it yields an enormous profit. It is difficult to stop this traffic since it contains the ordinary constituents of cows' milk and passes the ordinary tests of the Food Inspector. The percentage of water and cream can be regulated to a nicety. Whereas, however, the average fat content of genuine cows' milk is about 3.6% the manufacturers of these sophisticated milks are usually content to keep within the legal minimum of 3%; milk which yields consistently on analysis a fat content of 3% or thereabouts is open to grave suspicion. Another device is the mixing of skimmed milk, plus additional cream, with ordinary milk. The addition of one gallon of cream to 16 gallons of skimmed milk gives a liquid with fat content just below the minimum standard, but as genuine milk contains fat in excess of this standard the mixture of the two gives a fat content above the legal minimum but less than the average from genuine milk. This gives an additional profit of 9s. 6d. per churn. Present legislation is inadequate to deal with these new activities.—*Brit. med. J.*, ii/1934, 520.

See also Food Substitutes, p. 843.

CREAM

Cream was formerly obtained by skimming from milk which had been allowed to stand overnight. It is now largely prepared by the use of separators depending on centrifugal action. The milk is very effectually deprived of its fat, and the cream is correspondingly richer. There is no standard for the amount of fat, but cream so produced may readily contain 65% or more.

Under the **Milk and Cream Regulations of 1912**, the addition of preservative (which is now prohibited altogether) was not allowed to cream containing less than 35% of fat. The Ministry of Health Report for 1922 stated that "it is fairly generally accepted in this country that cream should contain 40 to 50% of butter fat, with about 5% of non-fatty solids" (Liverseege). The need for definite standards has long been felt, and it is of particular interest that the standing committee of the Council of Agriculture made special reference to this need in their report to the Council in 1934. The Committee recommended a scheme previously put forward, suggesting three standards: a 12% cream for breakfast or coffee cream; a 25% standard for fruit cream; and a 50% standard for thick or whipping cream.

The Committee also called for the enforcement of legal standards for the amount and quality of actual cream in all ice-cream sold.

Devonshire or clotted cream may contain 50 to 60% of fat.

Tinned cream usually contains less than 35% of fat. It is often marked "Thick Cream" but "the thickness is due to sterilisation, and not to a good proportion of fat." According to Liverseege ("Adulteration and Analysis of Foods and Drugs") for practical purposes it may be stated that fresh cream contains over 40% of fat, and tinned cream about 23%.

The fat in cream may be determined by the methods employed for milk, about 2 g. of cream being diluted with water. If the apparatus is available the Leffmann-Beam process provides a rapid method, which is described in "Aids to the Analysis of Food and Drugs" (Moor and Partridge).

The Gerber process can also be used for cream. The Werner-Schmidt, Gottlieb, and Adams' methods are all suitable.

Lerrigo points out (*Analyst*, 1928, 488) that many commercial samples of cream contain added water, and refers to the presence and detection of glycerin (*ibid.*, p. 335). A formula for the calculation of added water is given in the *A.O.A.C. Methods of Analysis* (3rd Ed., p. 225), based on the freezing-point determination.

$$W = \frac{\% \text{ serum in cream } (T - T^1)}{T}$$

where W = percentage of added water

T = freezing point of undiluted cream (-0.550°)

T^1 = observed freezing-point of given sample

$\% \text{ serum} = 100 - (\% \text{ fat} + \% \text{ protein})$.

If protein has not been determined it may be assumed to be 38% of the solids-not-fat.

The use of **preservatives in cream** is entirely prohibited by the Public Health (Preservatives, etc., in Food) Regulations, 1925.

The addition of sucrate of lime, gelatin, starch paste or other thickening substances is also prohibited.

"Viscogen" is a form of sucrate of lime which has been used for thickening, its detection depending on the percentage of lime in the ash together with tests for cane sugar.

Starch may be readily detected by iodine solution, and gelatin by means of Stokes' test (see p. 801).

Synthetic cream is a mixture of flour, dried egg-yolk, sugar and water, with a vegetable or hydrogenated oil. The flour and water paste is heated to 210°F. for 30 minutes and then cooled; the egg-yolk powder and sugar are added; the fat is melted separately, held at 145°F. and atomised under high pressure into the starchy paste. The whole mixture is further heated at 160°–170°F. for 30 minutes, homogenised, and then cooled to 40°F. over brine coolers.—*Lancet*, 1/1942, 173. (See also *Food Substitutes*, pp. 843, 845 and 848.)

ICE CREAM

Ice cream can be divided into three types, "cream ice" in which milk fat forms one of the main ingredients, "custard ice" made largely from corn flour or some starchy constituent, and "water ice" containing little more than water, sugar and a flavouring agent. There are two methods of manufacture, the "hot mix," employed by most large manufacturers, in which the raw materials are mixed hot, and the "cold mix," often used in small shops, in which an ice-cream powder consisting of skimmed milk powder and other ingredients in the correct proportions is merely mixed with milk and frozen. Custard ice, such as is often sold from hand barrows, must be brought to boiling point during manufacture and hence has usually been sterilised. Most manufacturers in their own interests require the mix to be properly pasteurised and ensure that subsequent storage and handling are conducted under conditions of strict cleanliness but many small dealers are content to use unpasteurised milk and their methods of handling and sale are far from satisfactory.

Bacterial content. Thirty-six samples of ice cream bought in a London district during the summer of 1934 were examined with the following results:—

The number of colonies per millilitre grown on agar at 37° in 48 hours ranged from less than 1000 to 4½ million (in one case).

4 samples contained over 1 million per ml. (including the above case)

2 " " less than 500,000 " but more than 500,000

10 " " " 500,000 " " 100,000

The remainder all contained less than 50,000.

Coliform bacilli were not found in 18 cases (1/100th ml.)

" " " present " 3 cases in 1/100th ml.

" " " " " 15 " 1/1000th ml.

" " " " " 6 of these cases in 1/10,000th ml.

" " " " " 3 " 1/100,000th ml.

the enumerations in these last three cases being 925,000; 308,000; 4,600,000.

The methods of examination and the media used corresponded with those prescribed by the Ministry of Health for Graded Milks, see p. 792.

Hasty deductions should not be drawn from the large numbers of bacteria in ice cream, for many may be the harmless lactic bacteria always met with in milk; a high count of *B. coli* may, however, justify a further enquiry into the manufacture and subsequent handling of this food.

Outbreaks of Food Poisoning Due to Ice Cream.

The Ministry of Health has records of 20 outbreaks due to ice cream, as follows: scarlet fever 1, typhoid 9, paratyphoid 2, dysentery 1, and 7 due to

organisms usually responsible for food poisoning.—*Rep. med. Offr Minist. Hlth, Lond.*, 1935, 138.

In Lichfield Borough 123 persons were affected with gastro-enteritis; all the infected persons had eaten ice cream manufactured by one firm. Infection with an organism of the coliform group was suspected, but the cause of the outbreak could not be definitely ascertained.—*Rep. med. Offr Minist. Hlth, Lond.*, 1936, 150.

Outbreaks of food poisoning, epidemiologically traceable to ice cream, occurred rather frequently aboard a large ocean liner sailing from San Francisco. Laboratory studies revealed excessively high bacteria colony counts in specimens of the ice cream. The cultures showed *Staphylococcus aureus*, which may have been the causative factor, to be the predominant organism. Since the lower temperatures used in ordinary refrigeration and ice cream freezing have only growth-inhibiting and not bacteria-destroying power, and since milk and milk food products, because of their inherent character, offer excellent bacteria culture medium possibilities, ice cream and other milk food products should be subject to rigorous standards in production, processing and marketing, with the provision of every reasonable safeguard to the public.—J. C. Geiger, A. B. Crowley and J. P. Gray, *J. Amer. med. Ass.*, ii/1935, 1980.

An outbreak of diphtheria due to eating ice cream occurred in Glasgow in August 1937, involving 13 persons, 6 of whom died. The shop-keeper who made and sold the ice cream was proved to be a virulent nasal carrier.—E. Bloch, *Lancet*, i/1938, 837.

See also *Food and Drugs Act*, pp. 845, 846 and 849.

BUTTER ANALYSIS

The general examination of butter includes determination of the proportions of water, curd, salt, and fat in the sample, followed by a special examination of the clarified fat for the presence of foreign fats or oils.

(i) **Water.** Heat 5 g. in an air-oven to 110°. The loss must not exceed 16%; if more, suspect careless making or intentional adulteration.

(ii) **Curd and Salt.** Melt the residue of (i) and treat with 10 ml. of ether, filter through a tared filter, repeat the process and wash until all ether-soluble matter is removed, dry the residue, and weigh: the residue consists of curd and salt.

(iii) **Ash.** Ignite residue from (ii) and weigh. Should be wholly salt; confirm this by titration with standard silver nitrate solution.

(iv) **Fat.** Should be taken by difference by subtracting the sum of percentages of water, curd and salt from 100.

(v) **Detection of Foreign Fats.** Butter-fat has certain characteristics which help to distinguish it from other fats. Some of its constituents are of lower molecular weight than those found in other animal and vegetable fats and oils. When the fat is saponified, the glycerides composing it yield glycerol and fatty acids which are liberated in acid solution. They have the marked odour of butyric acid, the most characteristic component of butter, and contain a larger proportion than usual of fatty acids soluble in water and volatile in steam, and this property is made use of in analysis for the detection of foreign fats. Besides butyric acid there are present caproic, caprylic, capric, lauric, myristic, palmitic, stearic and oleic acids, which may occur as simple glyceryl esters such as triolein or glyceryl trioleate, or as mixed

esters in which more than one fatty acid radicle is combined in the molecule of the glyceryl compound, for example, palmito-distearin. Compounds of oleic, palmitic and stearic acids preponderate in animal fats such as lard, tallow and beef-fat, and in varying proportions in most vegetable fats and oils. Coconut and palm-kernel oils are intermediate in character but yield considerably less soluble volatile acids, and an increased proportion of insoluble volatile acids in comparison with butter-fat. The introduction of these and other oils (see Margarine) considerably increased the difficulty of detecting adulteration. Formerly distillation of the fatty acids by the Reichert method, determinations of iodine values and other chemical constants, and physical characters such as solubility or miscibility and the refractive index all furnished clear indications of the presence of foreign fats, usually oleo-margarine, but, by skilful admixture of various fats and oils now available with butter of a high Reichert value, blends may be produced which answer many of the tests for genuine butter.

At the present day the combined Reichert-Polenské-Kirschner processes form the most important means relied on for the detection and estimation of the amount of adulteration.

The *Reichert* process has undergone modification in detail by Meissl, Wollny, Leffman and Beam and others to its present form.

The values obtained for 5 grammes of butter-fat are known as the Reichert-Meissl or Reichert-Wollny values, the saponification being carried out with the glycerol-soda solution, introduced by Leffmann and Beam, instead of alcohol, for the purpose of the Polenské determination which follows.

Carried out under standard conditions of method and apparatus, the combined process yields:—

(i) The *Reichert-Meissl Value* which is a measure of the soluble volatile fatty acids obtained from 5 grammes of fat.

(ii) The *Polenské Value* which is similarly a measure of the insoluble volatile fatty acids.

(iii) The *Kirschner Value* which practically measures the butyric acid in the sample.

The following solutions are required:—

(a) A strong solution of sodium hydroxide prepared by dissolving 100 g. of sodium hydroxide in 100 ml. of water and allowing to stand until clear, the solution being protected from access of CO_2 .

(b) Glycerol-soda: made by mixing 20 ml. of the sodium hydroxide solution with 180 ml. of glycerin.

(c) Dilute sulphuric acid: made by carefully adding 200 ml. of concentrated acid to 800 ml. of water.

Reichert-Meissl or Reichert-Wollny Value. Saponify 5 g. of the clear melted butter-fat in a 300 ml. flask by heating with 20 ml. of the glycerol-soda solution; allow to cool somewhat and add, cautiously at first, 135 ml. of hot water which has been well boiled. Add a few fragments of pumice, and then acidify with 6 ml. of the dilute sulphuric acid. Attach the flask to a standard distillation apparatus (*Methods of Analysis, A.O.A.C.*, 3rd Edn., p. 323; *Analyst*, 1904, 155) and distil so that 110 ml. of the distillate is collected in about 20 minutes. Remove the 110 ml. receiving flask and place a measuring cylinder (or other suitable vessel) under the condenser. Cool the distillate to 15° , mix gently, and filter the 110 ml. through a dry filter rejecting the first few millilitres. Titrate 100 ml. with N/10 soda using phenolphthalein as indicator. Set aside the titrated liquid for the Kirschner determination.

From the number of millilitres of N/10 soda required by the 100 ml. of distillate, deduct the amount required in a blank experiment conducted with the

reagents but without the fat, then add on one-tenth (110 ml. having been distilled) to obtain the Reichert-Meissl value.

Polenské Value. Wash the condenser tube, measuring cylinder and 110 ml. flask with small quantities of water, 18 ml. to 20 ml. in all, and pass the washings through the filter used in the Reichert process. Reject the filtrate. Dissolve the water-insoluble acids by passing three successive 15 ml. portions of neutral alcohol through the condenser, cylinder, 110 ml. flask and filter paper. Titrate the combined alcoholic washings with N/10 soda using phenolphthalein as before. The number of millilitres required is the insoluble volatile acid or Polenské value.

Kirschner Value. Add 0.5 g. of finely powdered silver sulphate to the titrated liquid from the Reichert determination, and shake frequently during at least an hour. Filter and transfer 100 ml. to a 300 ml. flask, add 10 ml. of dilute sulphuric acid (25 ml. per litre), 35 ml. of water, a little pumice, and distil in the standard apparatus, collecting 110 ml. in about 20 minutes. Titrate 100 ml. with N/10 soda, deduct the result of a blank experiment, and calculate the Kirschner value from the following equation.

$$K = \frac{110}{100} \times \frac{110A}{100} \times \frac{100 + B}{100} = \frac{121A(100 + B)}{10,000}$$

where A = corrected Kirschner titration

B = number of millilitres of N/10 alkali required to neutralise 100 ml. of Reichert-Meissl distillate.

Standard process in detail for the Reichert-Polenské-Kirschner values will be found in the *Analyst*, 1936, 404.

The Kirschner value is of importance in the detection and *estimation of butter-fat in margarine*, which must not contain more than 10%.

In a paper on the examination of New Zealand butter-fat by Hilditch and Jones (*Analyst*, 1929, 75) it is stated that "the relation of the observed butyric acid content to that calculated on the assumption that the Kirschner value is a simple measure of the butyric acid would seem to show that the latter registers in terms of butyric acid about 15 to 20% more than is actually present in the fat."

The possibility that the Kirschner value included caproic acid was mentioned in the discussion.

Reichert values for genuine butter-fat usually lie between 24 and 32, Polenské values range from about 1.4 to 3.5 and the Kirschner values from 20 to 26. These values are roughly proportional, a high Reichert being accompanied by high Polenské and Kirschner, whilst low values for one are associated in genuine samples with low values for the others.

The limits given above for the Reichert value are frequently exceeded and, in particular, values of 23 and lower are met with (in Irish, Russian and Siberian butters) which are not necessarily suspicious, especially if the Polenské and Kirschner correspond.

A British Standard Specification (B.S.S. No. 769—1938) has been issued by the British Standards Institution giving methods for the chemical analysis of butter. The specification describes the recognised methods used in the routine examination of all butters and the following supplementary special determinations are described in an Appendix:—Curd-protein, lactose, acidity, common salt, copper and iron, the iodine value, saponification value and refractive index of the fat, the peroxide oxygen in the fat and hydrogen-ion concentration of the curd-serum.

Coconut oil gives a Reichert-Meissl value of 6 to 8, and a Polenské of 15 to 20. It thus contains an appreciable amount of volatile fatty acids soluble in water though much less than butter-fat.

The high Polenské representing volatile insoluble acids is characteristic of both coconut and *palm kernel oil*, distinguishing them from butter and from other vegetable oils and fats. It is said to be due mainly to myristic and lauric acids (Winton: *Structure and Composition of Foods*, Vol. I, 384), other acids found being palmitic, stearic, oleic and caprylic, but not caproic and capric.

As in butter, it appears also in coconut oil that the esters are not necessarily simple triglycerides of one acid, but that two or three acid radicles may occur in one molecule, e.g., caprylo-lauro myristin and myristo dilaurin.

Various formulæ have been proposed for calculating the approximate composition of adulterated samples of butter from the analytical data. Different

formulae have to be used according to whether coconut and palm kernel oil are present or not.

Much work has also been done on the subject of the variation of the analytical figures obtained with known mixtures of butter and coconut oil from the calculated figures.

Experience in interpretation of the results of analysis is of great importance in this as in other branches of chemical work. A valuable guide to a decision may be furnished by the preparation and analysis of a series (or rather two series) of mixtures of butter-fat (*a*) of high and (*b*) of low Reichert values with different percentages of coconut oil and/or other possible constituents.

The possession of such analytical results of known mixtures provides a very useful check upon the conclusions arrived at by other means.

Application of the Phosphatase Test to Butter. About 30 g. of butter is melted at 40° in a centrifuge tube and centrifuged, and the aqueous layer is withdrawn by means of a pipette. In Test A, 0.5 ml. of this aqueous layer is used instead of 0.5 ml. of milk, the mixture is heated for 10 minutes at 47° and cooled, and the colour is developed as described (*see p.* 790). A blue of greater intensity than the standard (2.3 blue units) suggests a raw or poorly pasteurised cream. This has been confirmed with butter made from raw cream, and from cream pasteurised in the laboratory by the holder process at 145°F. for 30 minutes, and with butter made in New Zealand from raw cream, and from cream pasteurised by the flash pasteurisation method.—H. D. Kay and W. R. Graham, *J. Dairy Res.*, 1936, 191.

Vitamin Content of Butter. Australian and New Zealand butters sold in this country have as high a vitamin A and D content as butters produced in Great Britain and elsewhere in Europe. There is a considerable fall in the vitamin content of British butter during the winter, from stall-fed cows, but the vitamin content of Australian butter shows little decline. Cold storage has little effect on vitamin content—even after two years cold storage little loss can be detected; neither is it affected by the racial origin of the herds.—*Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 175, 1932; *Brit. med. J.*, ii/1932, 1024.

Vitamin D of butter is destroyed by boiling with alcoholic potash. In this respect it is different from the vitamin D of irradiated ergosterol and of cod-liver oil.—S. G. Kon and R. G. Booth, *Biochem. J.*, 1933, 1302.

The average vitamin A content of 75 samples of butter from various sources, determined by biological assay, was found to be 22.5 units per g., the figures ranging from 5 to 38.6 units per g. The vitamin D content averaged 0.36 unit per g. with a range of 0.06 to 0.99 unit per g. The average vitamin A and vitamin D contents of summer butter were 27.2 and 0.49 units per g. respectively; for winter butter the averages were 15.4 and 0.16 per g. respectively.—R. S. Morgan and H. Pritchard, *Analyst*, 1937, 354.

(*See also Vitamin Tables, p.* 769.)

Bacteriological Examination of Butter. 5 g. of butter is accurately weighed, transferred to a sterile mortar and pounded with 95 ml. of warm water. The mixing must be thorough as the bacteria adhere to the butter-fat. Dilutions are made, plates poured and colonies counted as for milk analysis. Ordinary butter may contain a million or more bacteria per g.

Tuberculous Butter. Persistence of tubercle bacilli in butter from tuberculous milk. Milk from tuberculous cows was made into butter and guinea-pig inoculations were made from the material. The milks were shown to contain tubercle bacilli and one sample of butter made from naturally ripened milk contained it. The butter made in other ways from this milk was similarly infected. The question of persistence of the organism in both salted and unsalted butter after ice storage is under further investigation.—H. A. Cookson, *Brit. med. J.*, ii/1926, 637.

The conclusions of the previous writer arrived at in U.S.A. in 1910. "Tubercle bacilli will retain their vitality and virulence while in butter, under common market conditions, for at least 5 months."—S. G. Moore, *Brit. med. J.*, ii/1926, 855.

Tubercle bacilli and *Br. abortus* may be found in hard or soft cheese immediately after manufacture but auto-sterilisation occurs during the ripening period. In the case of soft cheese the ripening period is short and the danger of tubercle bacilli surviving is correspondingly greater.—E. J. Pullinger, *Lancet*, i/1935, 1342.

Biological Detection of Tubercle Bacilli in Butter. From 60 g. to 100 g. of butter is melted at 40° in 150 ml. distilled water in a beaker. This is pumped through a cream-making machine into a centrifuge tube and centrifuged at 3000 revolutions per minute for 30 minutes. The cream is removed, the supernatant liquid poured off, the deposit suspended in 5 ml. of saline and inoculated subcutaneously in equal amounts into the artero-internal aspects of the right thigh of each of two guinea-pigs, the inguinal glands having been previously squeezed after the method of Block. The animals are killed after a month and examined for tuberculosis arising from the site of inoculation. Of 40 samples of Danish, New Zealand and Australian butters examined the organism was found in one of the Danish samples.—J. W. Eddington, *Lancet*, ii/1934, 81.

Keeping Qualities of Butter. Butter may be preserved by exclusion of air, addition of salt or of chemical preservatives. Most fat-hydrolysing bacteria are aerobic, therefore casks for the reception of butter are coated inside with paraffin wax, smaller quantities wrapped in parchment paper. Butter to be transported is packed in hermetically sealed tins. By these devices the development of surface-growing moulds is avoided and that of fat-hydrolysing bacteria is prevented.

See also *Food and Drugs Act*, p. 846.

CHEESE

Cheese may be defined as a food product, matured or not, prepared from milk, raw or pasteurised, cream or skimmed milk, derived from cows, ewes or goats. Details in the method of manufacture at all stages, especially in the ripening process result in a number of varieties of cheese, each possessing special characteristics; in France alone more than 300 kinds are officially recognised.

Manufacture. Almost all the cheese made in Britain and the Dominions is made from whole milk; a certain proportion of Stilton and Wensleydale cheeses is made from milk enriched with cream. Some varieties of Dutch cheese are made from skimmed milk; Roquefort and Parmesan cheeses are made from partly-skimmed milk obtained from ewes and goats respectively. Previously pasteurised milk is inoculated with a culture of mixed lactic acid organisms called a starter. Souring takes place and when the desired degree of acidity is reached, rennet is added to produce coagulation. After standing, the curd is cut into small pieces by means of special knives, thus liberating the whey, drained, thoroughly mixed by passing through a mill or by other means, salted and pressed. Finally the cheese is wrapped in cloth and allowed to ripen for some weeks in order to produce the characteristic flavour and taste. The mellowing of the cheese is largely controlled by the activity of proteolytic enzymes, the development of flavour and taste is chiefly due to lipase activity.

Composition and Analysis. There are no official standards for the composition of cheese. National Mark Cheshire Cheese must conform to the following specification:—the cheese shall be a hard-pressed cheese made from full-cream cow's milk and shall contain a minimum of 45% of butter-fat in the moisture-free substance. The figures for water and fat in English cheeses are summarised from the *Analyst*, 1941, 265.

| Type of Cheese | Number of Samples | Water | Fat | Fat on Moisture-free |
|------------------------|-------------------|-------|-------|----------------------|
| Caerphilly | 234 | 44.67 | 28.43 | 51.23 |
| Cheddar | 363 | 36.69 | 31.82 | 50.20 |
| Cheshire | 606 | 40.53 | 29.96 | 50.30 |
| Derby | 11 | 39.95 | 30.59 | 50.7 |
| Gloucester | 3 | 41.0 | 28.8 | 49.0 |
| Lancashire | 403 | 44.28 | 27.39 | 49.10 |
| Leicester | 24 | 39.79 | 30.21 | 50.0 |
| Stilton, White | 124 | 41.76 | 32.44 | 55.37 |
| „ Blue | 212 | 37.64 | 35.71 | 57.20 |
| Wensleydale | 111 | 45.03 | 27.72 | 50.23 |
| Cream | 49 | 24.50 | 71.60 | — |

The proximate analysis of cheese includes the determination of water, ash, fat and proteins. Owing to the uneven distribution of water, careful sampling is necessary. The fat which is similar to butter-fat in composition is examined by the Reichert-Kirschner-Polenské method (*see* Butter).

If the cheese has been prepared from whole milk the ratio fat:protein, will be not less than 1:1 and may reach 1.25:1. In cream cheese the ratio should be higher. The percentage of fat in the moisture-free cheese should not be below 50. Lancashire and Gloucester cheeses sometimes give lower figures and it is asserted that a certain amount of skimmed milk is used towards the end of the cheese-making season in making these varieties.

Richmond's formula for calculation of the composition of the original milk is as follows:—

Let F and P represent the fat and protein respectively in the cheese.

$$\text{Fat in the original milk} = \frac{100F}{35.4P + F} + 0.25$$

$$\text{Solids-not-fat} = \frac{333P}{35.4P + F}$$

Certain cheeses of the Gorgonzola type have been found to contain an excessive amount of rind, and occasionally the rind has been heavily weighted with barium sulphate.

A British Standard Specification (*B.S.S. No. 770—1938*) has been issued by the British Standards Institution giving methods for the chemical analysis of cheese. The specification describes the recognised methods used in the routine examination of all cheeses and the following supplementary special determinations, namely, hydrogen-ion concentration and titratable acidity, are described in an Appendix.

Water and fat content of 2140 samples of different varieties of cheese are given, together with the method for determining a factor "m," which factor is regarded as a slightly better criterion than the percentage of fat in the water-free substance of the cheese for determining whether or not a sample has been made from whole milk.—J. R. Nicholls, *Analyst*, 1941, 265.

Pasteurised or Processed Cheese is prepared by heating cheese usually with water, sodium citrate and an emulsifying agent to 80° and pouring the thoroughly mixed product into moulds. When set it is wrapped in tinfoil. Such cheese should

not contain an excessive amount of water nor any tin derived from the wrapper.

Two instances are described of processed cheese which was stated to contain glass. Examination revealed the presence of numerous transparent crystals of lactose resulting from the use of excess lactose in manufacture and loss of water by evaporation.—*Analyst*, 1939, 820.

Nutritive Value of Cheese. In Stilton cheese the percentages of calcium and phosphorus are much lower than in Cheddar and Cheshire cheeses due to differences in manufacture: the curd in the former is bathed in its own whey for several days and the whey drained without pressing; acidity develops and most of the calcium passes into the whey whilst a high proportion is found in the rind.

Vitamin A and carotene pass almost quantitatively from the milk to cheese and are retained practically entirely in the fully ripened cheese. About $\frac{1}{8}$ th of the vitamin B₁ is retained in the cheese and $\frac{1}{4}$ of the lactoflavin; the remainder is found in the whey. Vitamin C is present in the curd to a small extent but speedily disappears in the ripening cheese. The vitamin content of Stilton cheese is higher than that of Cheshire or Cheddar cheese.

English cheddar cheese was found in biological tests to have a vitamin A potency of an order (about 7.5 i.u. per g.) that would be expected from its milk fat content and from the result of colorimetric estimation of vitamin A and carotene. Colorimetric determination on other full-milk cheeses gave similar results. Lower values were found for cheeses of lower fat content.—A. W. Davies and T. Moore, *Biochem. J.*, 1939, 33, 1645.

See also *Vitamin Tables*, p. 769, and *Food and Drugs Act*, p. 848.

MARGARINE

Almost any animal or vegetable oil or fat may be used after deodorising, refining and if necessary hardening, provided that it can be obtained in sufficient quantity and at a sufficiently low price. In summer, fats of higher, in winter of lower melting-points are used. Formerly only animal fats were used (oleo-margarine) and the best qualities are still made from animal fats only but most margarine is now made from vegetable fats exclusively (principally from nut oils) or from a mixture of vegetable and animal fats.

Oleo-margarine. Premier-jus, the first runnings of fat obtained by heating the fatty tissues of the caul and kidneys of cattle at a temperature of 37° to 50°, is washed with brine and allowed to crystallise. The liquid oleo-oil is separated from the solid oleo-stearine, the oil being used in the manufacture of oleo-margarine. The fatty base of vegetable margarine is a mixture of solid fat and liquid oil. The fat may be a hydrogenated (hardened) oil, coconut or palm-kernel oil, the oil a mixture of bland oils such as cottonseed, arachis, shea-nut or soya.

Manufacture. Separated milk is pasteurised, inoculated with a suitable culture of the lactic acid bacillus, e.g., the Leichmann strain and maintained at a suitable temperature until soured. Separated milk is easier to control bacteriologically than whole milk and there are other objections to introducing butter-fat into margarine. The fats are melted in water-jacketed pans, annatto

or other colouring matter added, run into the mixing pans containing the prepared milk and the mixture churned until of the right consistency. The product is further treated either by running over brine-cooled large rollers, or projecting against a spray of ice-cold water. In either process the product obtained is thoroughly kneaded, salt and any other ingredients being incorporated. During emulsification, vitamins A and D are introduced in proportions similar to those in summer butter. In some countries a small percentage of sesame oil must be incorporated in the fat mixture in order to provide a simple test distinguishing margarine from butter. Traces of diacetyl, a natural ingredient of butter, are sometimes added to margarine to improve the flavour.

Analysis. It is almost impossible to distinguish between some brands of margarine and butter by taste, and special provisions of the Food and Drugs Act have been enacted to prevent adulteration of butter with margarine. Margarine may not contain more than 16% of water and the fat of margarine more than 10% of butter-fat. The percentage of butter-fat in margarine can be determined with considerable accuracy by analysis, but the identification and amounts of the various fats which may be mixed in a sample of margarine is a matter of great difficulty. (*For methods of analysis, see Butter, p. 808.*)

Nutritive Value of Margarine. Margarine and butter have much the same food value. According to the 1933 report of the Nutrition Committee of the B.M.A. the calorie value of margarine is about 2% higher than that of butter. Some brands of margarine containing vitamins have been on the market since 1927. Since 1940, in accordance with the Ministry of Food requirements, all margarines sold to the public contain these vitamins. The potency was about 20 i.u. of vitamin A and 1 i.u. of vitamin D per g, but early in 1942 the vitamin D content was doubled. Vitamin A is added in the form of a liver oil concentrate, and vitamin D is added as calciferol.

Determination of Vitamin Content. Spectrophotometric assay of vitamin A with special reference to margarine.—J. R. Edisbury, *Analyst*, 1940, 484. (*For further details, see p. 733.*)

Margarines of vitamin D potency 1 i.u. per g. can be assayed satisfactorily by the normal line-test method. Assays of the vitamin concentrates and the margarine made with them show that the margarine assay is not falsified by the obligatory feeding of fat to the test animals. The assay of very low potency margarines, however, entails the feeding of larger quantities of fat, which have been found to affect the responses of the test animals. The more sensitive bone-ash method has been suggested as a preferable method. This has been tested but the two methods give somewhat different results. It is possible that the international standard is not so effective in the line-test as it is in the bone-ash assay. Results indicating such a difference in activity have been obtained.—N. T. Gridgeman, H. Lees and H. Wilkinson, *Analyst*, 1940, 493.

FLOUR, BREAD AND OTHER STARCH FOODS

Civilised man, living a normal life, derives a large portion of his nourishment from cereal foods. The Anglo-Saxon, the Latin and

some of the Teutonic races use wheat flour or meal as their main cereal food; a portion of the Teuton races and many Eastern peoples use a considerable quantity of rye flour. Rye will grow on poorer and lighter land than wheat and will withstand greater extremes of temperature. It is, however, lacking in gluten-forming proteins which are essential to the formation of a loaf of bread having a good volume and smooth, porous texture. Bread made wholly from rye is small in volume, compact and somewhat soggy. Kent Jones and Bacharach calculate that baker's bread provides the British nation with over 20% of its total calories and over 20% of its protein. When other flour products (home-made bread, biscuits, cakes, etc.) are taken into account, wheat provides over 35% of the total calories and about 35% of the proteins.

Constituents of Wheat. The wheat berry consists of an outer shell or pericarp (4%), a seed coat (9%) consisting of testa, hyaline and aleurone layers, a small germ (2%) and a central mass called endosperm (85%). The pericarp and the layers of the seed coat together constitute the bran, but, as commercially separated, bran is always accompanied by varying amounts of endosperm (up to 40%).

Typical analytical data for wheat and its commercial fractions are given by R. G. Booth, R. H. Carter, C. R. Jones and T. Moran (*J. Soc. chem. Ind., Lond.*, 1941, 60, 903):—

| | Whole Wheat | Endosperm (75% extraction) | Bran | Germ |
|---------------------------------|-------------|----------------------------|-------|------|
| Moisture, % | 12.2 | 13.8 | 12.5 | 13.5 |
| Oil, % | 1.8 | 1.2 | 4.7 | 10.8 |
| Proteins (N \times 5.7), % .. | 13.2 | 12.0 | 15.0 | 25.9 |
| Fibre, % | 2.1 | 0.2 | 7.9 | 1.9 |
| Ash, % | 1.7 | 0.55 | 4.65 | 4.1 |
| Carbohydrates, % .. | 69.0 | 72.25 | 55.25 | 43.8 |

(There is a considerable range in composition according to variety, climate and cultural conditions.)

Milling. The entire wheat grain may be crushed to produce wholemeal; sometimes about 5% of the coarsest bran is excluded. More usually the grain is subjected to a series of grinding and bolting processes whereby the branny portions are separated from the flour. Most of the flour is not made directly but by regulated crushing or rolling the endosperm passes through the stage of semolina. Various sizes of semolina are produced, some free or easily freed from bran and some contaminated with more or less bran. When these semolinas are reduced to flour by subsequent rollers, there are several flour streams of varying degrees of purity. Straight-run flour consists of all these flour streams mixed together but flour streams may be segregated to produce high-grade or low-grade flours. The percentage of the wheat appearing as flour is called the extraction. It is impossible in the milling process to

remove all the constituents of the endosperm, leaving the bran separate; similarly there is always floury matter attached to the bran. The higher the extraction the more fine bran goes into the flour. The maximum amount of highest grade white flour which can be produced is about 70%. In normal times the average extraction of white flour is about 72%; in 1941 the extraction was compulsorily fixed at 75% and only straight-run flour was permitted; in 1942 National Wheatmeal specified 85% extraction and as much as possible of the germ. The composition changes according to the extraction, as may be seen from the figures given below for straight-milled flours from the same grist (R. G. Booth, R. H. Carter, C. R. Jones and T. Moran, *J. Soc. chem. Ind., Lond.*, 1941, 60, 903).

| Material | Ash % | Protein % | Vitamin B ₁ i.u. per g. | Fibre % |
|------------------|----------|--------------|---------------------------------------|------------|
| 70% flour | 0.445 | 11.33 | 0.25 | — |
| 75% " | 0.517 | 11.60 | 0.42 | — |
| 80% " | 0.689 | 11.90 | 0.77 | 0.3-0.4 |
| 85% " | 0.892 | 12.06 | 0.85 | 0.6 |
| 100% wheat | 1.64 | 12.72 | 1.3 | 2.0 |

FLOUR

Flour has been graded into many types such as patents, supers, bakers, household, straight-run, etc. Patents and supers consist of only a proportion of the whole of the flour made and represent 70% extraction or less of very white flour.

The Ministry of Food in 1942 raised the extraction to 85% largely because of tonnage and not on nutritional grounds. At the same time it was defined in such a way that the most nutritional product should be available. Statutory Rules and Orders, 1942, No. 451 specifies "National Wheatmeal" means in relation to flour produced after the 22nd day of March, 1942, flour of 85% extraction, produced in the United Kingdom, so as to contain the maximum quantity of wheat germ which, having regard to the type of milling plant, can be included in such flour, but so as not to include any coarse bran; provided that national wheatmeal does not include flour so produced to which has been added any substance other than a substance authorised by the Minister to be an ingredient of national wheatmeal." The basis of this 85% flour is that it should contain: (1) the maximum of germ, (2) the maximum of the aleurone layer, (3) the minimum of fibre.

The Research Association of British Flour Millers describes the product as including all the white flour, all the reduction roll tails, mill finish and purifier tails, and sufficient fine bran to bring the amount of the mixture to 85% of the clean wheat.

It is possible to produce flour of 85% extraction with a very low fibre and yet containing a high vitamin B₁ content. Most mills cannot do this without modification of milling technique, since it

involves scraping but not cutting the bran. It is the bran which supplies most of the undesirable phytin phosphorus and this is one reason why it is required that the fibre content of national wheatmeal shall be as low as possible.

Wheatmeal of 14% moisture will keep in excellent condition for 9 months. At 15½% moisture the maximum life is 2 months. Wheatmeal is more prone to insect infestation than white flour.

National Mark Flour (in abeyance since 1939) included:—

1. **ALL-ENGLISH (WHEATMEAL)** which shall comprise at least 90% of the ground products of the wheat; no bran shall be added thereto or flour extracted therefrom. Ash content 1.0 to 1.70% and fibre content 1.0 to 2.0%, both calculated on the basis of 15% moisture.

2. **ALL-ENGLISH (STRAIGHTS)** which shall comprise all the flours obtainable from the wheat. Ash content shall not exceed 0.52% at 15% moisture.

3. **ALL-ENGLISH (PATENTS)** which shall be as produced at the head end of the mill from "purified" stock. Ash content shall not exceed 0.45% at 15% moisture.

4. **ALL-ENGLISH (SECONDS)** which shall comprise the residue of the flours not otherwise separated as Patents. Ash content shall not exceed 0.62% at 15% moisture.

5. **ALL-ENGLISH (SELF-RAISING)** which shall comprise Straights or Patents along with suitable raising ingredients.

6. **WHEAT FLAKES** which shall comprise the whole wheat berry without the addition or extraction of bran or flour. Any addition of malt and/or sugar shall be declared on the container.

Self-raising Flour is flour containing the necessary constituents for the production of gas, generally baking powder, the quantity added being about 3%.

Baking Powder consists of an intimate mixture of carbonates, acids and fillers. The carbonate normally used is sodium bicarbonate and the acid either an acid phosphate (acid calcium phosphate or sodium acid pyrophosphate) or tartaric acid or an acid tartrate (cream of tartar). The filler is some form of starch or flour which coats the particles and thus prevents premature reaction. In some parts of the world aluminium compounds are used as an acid ingredient, but objection is often taken to them in this country. The acid and carbonate ingredients are present in approximately equivalent proportions and the amount of filler is about 50%.

Nutritional Qualities of Flour.

(a) **Proteins.** The biological value of whole-wheat proteins is higher both in protein-sparing and in growth-promoting effects than that of low extraction flours. Whether this is to be attributed solely to the nature of the proteins is uncertain.

(b) **Carbohydrates.** Starch is the predominating carbohydrate; there is no appreciable amount of sugar.

(c) **Oil.** The oil is small but may be of nutritional importance in connection with vitamin E.

(d) **Minerals.** The chief inorganic constituents are potassium, phosphorus, sulphur, magnesium, chlorine and calcium. Phytin phosphorus constitutes about 75% of the total phosphorus in whole wheat, 90% in bran, 45% in germ, 60% in wheatmeal (85% extraction) and 35% in white flour (75% extraction). The iron content, although small, makes an appreciable contribution to the daily requirements because of the quantities of flour consumed in one form or another; this is especially true of the higher extraction flours.

The iron of brown bread is not nearly so available to the body as that contained in white bread.—McCance and Widdowson.

Flour is not an important source of calcium, contributing only about one-tenth of the daily requirement of 0.6 g. There is much evidence of calcium deficiency in many diets and flour appears a convenient vehicle to make it up. The ratio of calcium to phosphorus in white or wholemeal flours is 1:6½-10 and is, therefore, not well suited to the needs of the animal body which are best met by foods where the ratio is 1-2:1. This is an argument against the addition of calcium phosphate, although it would tend in some degree to correct the balance. The addition of calcium carbonate at the rate of ½ lb. per 280 lb. of flour would raise the calcium intake of Sir John Orr's lowest income group to roughly the minimal level. This quantity of calcium carbonate has no adverse effect on the palatability or general quality of bread.—Moran and Drummond, *Nature, Lond.*, 1940, 117.

(e) **Vitamins.** Wheat contains no appreciable amounts of vitamins A, C and D. The colouring matter until recently was regarded as carotene, a precursor of vitamin A; it is now known to be, for the most part, xanthophyll. Vitamin B₁ (aneurine) is present in significant amounts and with it is associated the other members of the B₂ complex, viz., riboflavin, nicotinic acid, pyridoxine and pantothenic acid. Wheat germ is a rich source of vitamin E (tocopherol), containing about 0.3 mg. per g.

The amount of vitamin B₁ increases with the extraction:—

| | |
|----------------|-----------------|
| Patent flour | very low |
| 70% extraction | 0.3 i.u. per g. |
| 75% " | 0.5 " |
| 85% " | 1 " |
| Bran " | 2 " |
| Germ | 8 " |

Straight-run white flour (73% extraction) has a B₁ content of 0.3 i.u. per g., whereas 85% extraction flour has 0.75 and wholemeal (100%) has 1.3. White flour contributes 80 i.u. per day and national wheatmeal 180 i.u.—Moran and Drummond, *Nature, Lond.*, 1940, 117.

Determination of Vitamin B₁. The process for the determination of aneurine in white flour (*Analyst*, 1942, 67, 15) with slight modification has been found suitable for National Wheatmeal.—R. G. Booth, *Analyst*, 1942, 162.

Determination of Nicotinic Acid. A colorimetric method based on the Mehrick-Field method (*J. biol. Chem.*, 1939, 130, 97) is described. Whole-wheat flour contains from 5.2 to 6.5 mg. of nicotinic acid per cent.; patent flour from 1.3 to 1.9 mg. per cent. The more flour is refined, the lower its nicotinic acid content. During the fermentation and baking of bread, only small losses occur.—D. Mehrick *et al.*, *Industr. Engng Chem. (anal. Edn.)*, 1941, 879.

Bleaching of Flour. Flour bleaching originated largely in an attempt to satisfy the demand for a very white flour. It removes the yellowness due to carotinoids (xanthophylls) but hardly affects the colour of bran and dirt; it does not give to a low grade product the brightness which is characteristic of high grade. Natural flour which has been kept for several weeks has enhanced baking qualities and becomes bleached due to the oxidising action of the air, and artificial bleaching is claimed to accelerate a natural process, thus avoiding costs of storage.

The bleachers commonly used are:—(1) chlorine and nitrosyl chloride; (2) nitrogen trichloride; (3) nitrogen peroxide; (4) benzoyl peroxide.

Detection of Bleaching. A general test is that of Kent Jones and Herd (*Analyst*, 1927, 443), whereby the flour is shaken with light petroleum and the colour of the filtered extract is compared with standard colours. Unbleached flour gives at least a minimum depth of colour.

Chlorine and its Compounds. Flour is extracted with light petroleum, the solvent evaporated, the residue treated with alcoholic soda and ashed, the chlorine being then determined. Natural flour does not yield more than 2 parts per million of chlorine.

Nitrogen Peroxide. The use of this bleacher will leave residual nitrites in the flour. The sample is shaken with nitrite-free water and filtered. The filtrate is tested with Griess-Ilosvay reagents ((a) solution of sulphanilic acid in dilute acetic acid, and (b) a solution of alpha-naphthylamine in dilute acetic acid). In the presence of nitrites a pink colour develops. Unbleached flour may contain a nitrite-reacting substance, equivalent to not more than 2 parts of sodium nitrite per million.

Benzoyl Peroxide. A flour treated with this substance may contain unchanged benzoyl peroxide and also benzoic acid produced after reaction. The unchanged peroxide may be converted into benzoic acid by means of sodium hydroxide in the presence of acetone and the total benzoic acid can be oxidised to salicylic acid and be determined colorimetrically.—J. R. Nicholls, *Analyst*, 1933, 4.

A Departmental Committee of the Ministry of Health considered the whole question of the addition of substances to flour and in the Report (1927) a number of recommendations were made but none of these has been given legislative sanction. The Committee considered that the purity and wholesomeness of flour should be jealously guarded against unnecessary treatment with foreign substances, but they were not prepared to recommend the complete elimination of the bleaching agents and improvers now in use. Their view was that it should suffice to limit the use of these substances to those which appeared least open to objection and they thought that chlorine, nitrogen trichloride and benzoyl peroxide should not be amongst them. They also thought that declaration of such additions should be compulsory.

Improvers. These substances are added to flour with the avowed intention of improving the baking quality of the flour. Their use followed an attempt to accelerate the natural ageing of flour, which has long been known to enhance baking properties.

The most common improvers are acid calcium phosphate, dicalcium phosphate, potassium or ammonium persulphate and potassium bromate. These may be separated by shaking the flour with chloroform or carbon tetrachloride; the flour floats and the mineral additions sink. Some bleaching agents, such as chlorine and its compounds, are also improvers.

In 1913 a firm of millers was prosecuted under the Food and Drugs Act for selling flour that was not of the nature, substance and quality demanded, and to the prejudice of the purchaser in that it contained 63 parts of potassium persulphate per million parts of flour. After considerable expert evidence both for the prosecution and the defence, in which there was direct conflict of opinion, the case was dismissed, the stipendary holding that the addition of improvers does not prejudice the purchaser seeing that it actually improves the flour, is not put in to conceal inferior quality and is introduced in such small quantity as to be wholly innocuous in its effects.

The National Association of British and Irish Millers, Ltd. (Report of the Departmental Committee on the Treatment of

Flour with Chemical Substances, 1927) suggested the following limits:—

| | Amount per cent. |
|--|------------------|
| Extracts of malt and germ | no limit |
| Acid phosphate of calcium | 0.3 |
| Acid phosphate of ammonium | 0.2 |
| Persulphates | 0.04 |
| Chlorine | 0.07 |
| Bleaching with peroxide of nitrogen; nitrites as sodium nitrite for English trade | 0.0006 |

The Medical Research Council in 1941 expressed the opinion that the addition of certain improvers to bread baked from flour of high extraction facilitates the work of the baker and results in a more attractive loaf. Such evidence as is available does not justify at this stage the prohibition of their use in the baking industry.

Physical treatment of flour by heat and water vapour can produce considerable improvement in baking qualities.

Fortification of Flour. In 1941 The Medical Research Council reported that bread made from wholemeal or from flour of 85% extraction contains more calcium than white bread and also more phosphorus. A large proportion of the phosphorus, however, is present in a combination (phytic acid) in which it is not directly available to the organism, and at the same time diminishes the availability of the calcium present in the bread and in the rest of the diet. Recent tests made on human subjects for the Medical Research Council have shown that to maintain a sufficient supply of calcium salts for health, more calcium salts must be added to the diet. The most convenient way to do this is to add calcium carbonate to the wheat flour used for bread. As a result of the above tests on human subjects it has been calculated that additions are needed of 65 mg. of calcium per 100 g. of white flour (7 oz. of CaCO_3 as *Creta Præparata*, B.P., per 280 lb. sack) and of 130 mg. of calcium per 100 g. of flour of 85% extraction (14 oz. of CaCO_3 per sack). In the case of the 85% extraction flour the extra 7 oz. per sack is required to neutralise the effect of the extra phytic acid contained in the flour of this degree of extraction. It has long been a practice in baking to add similar amounts of improvers (e.g., acid calcium phosphate 12 oz. per sack, or up to $4\frac{1}{2}$ lb. per sack in the case of certain self-raising flours at present on the market). The addition of the recommended amounts of calcium carbonate to flour has no effect on the vitamin B_1 content of the flour or on the baking properties.

The ordinary baking process destroys at least half of the phytic acid; all the phytic acid can be destroyed by increasing the acidity of the bread to a pH of about 5.3. *Creta Præparata* has no effect on the amount destroyed.—Pringle and Moran.

Creta Præparata increases the pH too little to have any effect on rope development of practical significance.—Halton, *J. Soc. chem. Ind., Lond.*, 1942, 77.

Objections have been raised to the addition of calcium carbonate because it might give rise to stones in the kidney and other diseases, but these views have no experimental backing.

The Medical Research Council considered the desirability of adding iron in some form to flour, but in view of the possibility of the destruction of other nutrients present did not recommend its addition until further knowledge is available.

In 1941 the Ministry of Food introduced a vitamin B₁ flour which contained 0.2 g. of pure aneurine hydrochloride (vitamin B₁) per sack of 280 lb. white flour. Since white flour contributed about 80 i.u. of vitamin B₁ per day, the vitamin B₁ flour contributed about 180 units per day. With the compulsory production of higher extraction flours the vitamin B₁ flour disappeared.

In America flours are fortified with vitamins of the B₂ complex in addition to vitamin B₁. Iron is also added to an extent not exceeding 1.5 mg. for each 100 calories.

Enrichment of white flour with thiamin alone improves the quality of the "poor" diet, but enrichment of the flour with both thiamin and riboflavin improves the diet yet further. Flour must be enriched with nutrients other than thiamin and riboflavin to obtain a flour of nutritive quality comparable to that of whole-wheat flour.—M. Pewters *et al.*, *Proc. Mayo Clin.*, 1941, 426.

Examination of Fortified Foods. The biological, microbiological, chemical and physical methods for the determination of added vitamins to articles of food are reviewed and emphasis is given to the inherent virtues and defects of the different techniques.—H. E. Monk, *Analyst*, 1942, 83.

BREAD

Bread is normally made by mixing and fermenting flour, water, salt and yeast, with possibly other ingredients such as sugar and fat. Typical proportions are:—flour 280 lb., salt 4 lb., compressed yeast 2 lb., with sufficient water to make a dough. Less yeast may be used if a longer fermentation is allowed. Although flour is a highly nutritious substance it is not readily acted upon by the digestive juices unless in a suitable form. The use of yeast or other leavening agent in a proper manner ensures a digestible and palatable product. Yeast is therefore a most important ingredient.

Bread may only be sold by net weight and, subject to certain exceptions, no loaf shall be sold unless its weight is one pound or an integral number of pounds.

There are various types of bread:—

White bread is made with white flour and as sold usually contains rather more than 30% of water.

Wholemeal or Graham bread is made from the entire wheat berry. Some of the flours used are very fine, but others are more or less crushed wheat.

National Wheatmeal bread (1942) is made from flour of which at least 75% is national wheatmeal and in the manufacture of which no other ingredients than the following have been used: National flour, oil and/or fat, water, salt, yeast, improvers of the nature of yeast food, acetic acid, vinegar, acid calcium phosphate, sodium pyrophosphate, and sodium acid sulphate.

Rye bread (1942) is bread of which not less than 25% of the total flour content is rye flour and rye meal and at least 75% of the wheat flour content is of not less than 85% extraction.

Standard bread is made from flour with an 80% extraction. This was recommended by The Royal Society Committee in 1917 as being the most economical distribution of wheat between its use as flour for human food and as offal for feeding livestock.

Malt bread utilises a proportion of malt extract in its preparation.

Germ bread is made from flour containing additional germ, sometimes considerable proportions.

Soda bread is aerated by the action of sour milk assisted by sodium-bicarbonate.

Vienna bread is baked in special ovens in an atmosphere of steam.

Gluten bread is prepared for diabetic patients. Flour is made into a stiff dough and by kneading in water the starch goes into suspension, leaving a residue of gluten which may be used for baking or may be dried at a low temperature to a light brown powder. Gluten bread should contain about 5% of starch, but often it contains considerably more.

Brown bread. The modern types of brown bread date from the introduction of the roller-mill white flour about 1880. Most are made from ordinary white flour mixed with an appropriate proportion of bran; some contain a proportion of germ. Brown bread contains nearly 40% of water.

White Bread v. Brown Bread. The controversy between white and brown bread has raged for a number of years. It is essentially a battle between nutritionists on the one hand and millers on the other. It has long been known that white bread is deficient in nutritional qualities compared with brown but the public has always preferred white bread. In pre-war days nearly 95% of the bread eaten was white. In the early nineteenth century alum was almost universally used, partly to increase whiteness, but its use was discredited and it gradually ceased to be added. The introduction of roller milling facilitated the production of a very white flour and enabled the germ to be separated, thus removing the dull colour due to this constituent and one cause of deterioration of the flour on keeping. The craze for whiter flour was encouraged by the millers who adopted bleaching methods to destroy the naturally occurring pigments and thus hasten a process which normally occurs on storing flour.

The arguments brought forward by the advocates of white flour may be summarised as follows:—

1. Brown bread is unpalatable to the public and people will not eat it even if the price is reduced to that of white bread.
2. Brown flour will not keep so well as white and needs turning over more frequently.
3. Increase in the extraction reduces the amount of offals, germ and brans which are almost the most important items in feeding-stuffs for cattle and lack of them would jeopardise milk supplies.
4. Brown bread contains more water than white and this counter-balances the slightly higher protein content of brown flour. White bread yields 260 calories per 100 g; wholemeal bread 229 calories per 100 g. (McCance and Widdowson).
5. The branny particles pass through the gut substantially unchanged. White bread has a digestibility of 96% and wholemeal of 87% (Macrae, Bacon, Hutchinson and McDougal).
6. Increase of bran increases the phytin phosphorus which immobilises calcium.

This is an imposing list for nutritionists to counter and until recent years lack of precise knowledge of all the factors has militated against adequate reply. It now appears clear that the question is mainly one of the loss of essential vitamins, particularly B₁. Beri-beri is rare in this country but the effects of partial deficiency, including loss of appetite and physical vigour, and

disorders of the alimentary tract, the nervous system and cardiovascular system are believed by many to be widespread.

A daily ration to five healthy subjects containing half the minimal dose of B_1 resulted within 5 days in 4 of the subjects showing characteristic signs of B_1 deficiency, including fatigue and lassitude, anorexia, precordial pains, burning in the feet, muscle cramp and palpitation. An increased amount of aneurine in the diet caused all the symptoms to disappear within 3 days.—Jolliffe (U.S.A.).

There is not the same evidence that people are deficient in the members of the B_2 complex or in vitamin E. As regards mineral constituents the relative merits of the two flours are somewhat uncertain because of the higher phytic acid content of brown flour.

Bread is the staple food of poverty, less being eaten when people can afford meat and sugary dishes. The lack of vitamin B_1 in white bread is therefore of particular importance in the lower income classes. While 85% extraction wheatmeal has most of the virtues of wholemeal and is more attractive, it still contains more bran than will satisfy the public in normal times.

The solution to the controversy would appear to be for the millers to produce a flour of such composition that it contains sufficient vitamin B_1 to satisfy the nutritionists, and as low a fibre as will satisfy the people who desire white bread. Some progress has been made in this direction, particularly since the recent discovery that vitamin B_1 in the germ is ten times as concentrated in the scutellum half as in the embryo half. When wheat is conditioned before milling the germ subsequently separates as a whole and is difficult to incorporate in the flour, but by a process of dry milling the embryo can be removed as a unit, the scutellum adhering to the endosperm and being readily pulverised with the flour. The product without the embryo thus contains nearly all the vitamin B_1 of the germ.

When it is known that about two-thirds of the total amount of vitamin B_1 is in the bran and that the bran can be quite easily winnowed from the endosperm it is evident that ever since the dawn of history man has not been getting his full 45 or 50 i.u. of vitamin B_1 in each ounce of wheat he has eaten, nor has he been getting even close to that amount. There are many who regard white flour as a comparatively modern product, whereas it is known that the bread in common use in London towards the close of the 18th century was made from flour representing only about 55% of the wheat milled. It is almost certain that our grandfathers ate bread which contained very little more vitamin B_1 than the bread of to-day. Certainly it could not have contained more than half the vitamin B_1 of the whole kernel. The fact is that sifting the bran and the germ from the endosperm, no matter whether separated by rolls, mill-stone or the quern, will remove a large proportion of the vitamin B_1 present in the kernel. Our present flour contains about 25% of the total vitamin B_1 and it would be necessary to grind about 40% of the bran into the flour in order to get half the vitamin B_1 in the flour bag. It is said that the reason for the better nutritive value of "old-time" flours was that the germ was not "milled out" of the flour. Anyone who has actually tried to mill the germ "into the flour" will not be hasty in supposing that much of it was ever left in any flour that had been sieved. Even if it could all be milled into the flour the vitamin content would be raised only from about 0.4 i.u. to about 0.7 i.u. per g. and the remaining 60 or 65% would still go into the offal bags.—C. E. Rich, *Chem. & Ind.*, 1941, 611.

See also *Vitamin Tablets*, p. 768.

The Staling of Bread. On keeping, bread grows stale, some loaves remaining fresh longer than others according to how they are made and baked. Staling is indicated by softening of the

crumb, hardening of the crust and by general deterioration. While all the factors causing staling are not known, it is not due to simple loss of water but is a result of a combination of chemical and physical changes, largely associated with the gelatinised starch. Baking technique can control staling to a considerable extent, and a good craftsman can produce an excellent loaf which will remain fresh for a reasonably long period.

Diseases of Bread. The interior of a loaf is not completely sterile and spores of bacteria and moulds resist destruction at the baking temperature. Souring is produced by lactic bacteria, and moulds may grow often producing coloured areas. Rope is due to micro-organisms and is liable to develop in moist, warm conditions when there is a low degree of acidity. This disease produces a distinctive, sickly-sweet odour and sticky, brown spots. In extreme instances the middle of a loaf may become a strongly smelling, semi-fluid, brownish mass capable of being pulled into stringy threads. It is controlled by proper making, cooking, cooling and storage of the bread; improvers which keep up the acidity, such as acid calcium phosphate, are the best preventatives.

Flour Confectionery

Biscuits are made from flour and baking powder with variable quantities of fat, sugar and other special ingredients.

Buns and Cakes contain flour and sugar and there are usually included eggs, shortening (fat), salt, milk, flavourings, colour and raising ingredients. The lightness of cakes depends on raising ingredients which, like baking powder, produce gas or which, like beaten eggs or creamed shortening and sugar, trap and retain a considerable number of air bubbles. Fat gives tenderness and has a shortening effect; butter has a great and deserved reputation in this respect. The chief sugar used is sucrose, but liquid glucose, golden syrup, treacle or honey are also employed.

Other Starch Foods

The compositions of the other principal starch foods are:—

| Description | Water | Protein | Oil | Ash | Fibre |
|---------------------|-------|---------|-----|-----|-------|
| Arrowroot | 15 | 0.4 | Tr. | 0.1 | Tr. |
| Barley, pearl | 13 | 9.0 | 1.0 | 1.0 | 0.5 |
| Cornflour | 12 | 0.5 | Tr. | 0.2 | Tr. |
| Oatmeal | 8 | 14.0 | 9.0 | 2.0 | 2.0 |
| Potatoes | 75 | 2.0 | 0.1 | 1.0 | 0.5 |
| Rice | 12 | 7.0 | 1.0 | 0.5 | 0.4 |
| Sago | 13 | 0.3 | 0.2 | 0.2 | 0.1 |
| Tapioca | 13 | 0.4 | 0.1 | 0.1 | 0.1 |

Arrowroot is a prepared starch from the pith of the roots of *Maranta*. It is a light, white powder, which is insipid and inodorous, and remains sweet for long periods if kept dry. When mixed with boiling water it swells up to a jelly-like consistence, suitable for use as a demulcent. It is almost wholly starch.

Pearl Barley is prepared by grinding barley in a wide-set mill whereby the grains are rounded and polished. The whole of the husk is thus removed except that portion left in the furrow of the seed. Pot barley or Scotch barley is a smaller barley treated similarly but not so perfectly, so that only the outer cuticle is

removed. Before use pearl barley should be washed with water and the washings rejected. This will remove any facing material (see Rice). Barley has a somewhat similar composition to wheat and the amount of vitamin B₁ present is of the same order. Ninetenths of this vitamin is removed in the production of pearl barley.

Cornflour is a starchy flour made from maize by removing some of the protein and a good deal of the fat by treatment with alkalis. It behaves with boiling water similarly to arrowroot.

Oatmeal is the ground and dried kernel of oats. Its nutrients are similar in amount to wholemeal wheat, except that the quantity of oil is considerably higher. The bulk of oatmeal is consumed as porridge and during a 30-minute boiling about 10% of the vitamin B₁ present is destroyed. Many of the oat products sold as breakfast foods are partially prepared by the manufacturer, and while they are more convenient, there is no evidence that they are nutritionally better than oatmeal.

Potatoes. Owing to a different morphological form and to a high content of water, potatoes differ considerably from other raw starchy foods. The cereals and the prepared starches may be regarded as masses of dry nutrients which change little after maturing, and which can be stored for long periods without substantial modification. Potatoes are edible long before they reach their maximum size and growth changes occur after they have been lifted from the ground. The skin of the potato is thin. The interior is not completely uniform, but there is little difference in general composition between peeled and unpeeled potatoes. Of the dry matter less than 10% is protein, less than 5% is mineral matter and over 80% is carbohydrates, principally starch. A small proportion of sugars is present and during storage some starch changes to sugar and some sugar to starch, while respiratory action breaks down sugars to carbon dioxide and water. Freshly dug potatoes have valuable antiscorbutic properties due to the presence of ascorbic acid. The concentration of ascorbic acid reaches a maximum in August and generally amounts to about 30 mg. per 100 g., although certain varieties may reach 40 mg. During storage the amount gradually falls and at the end of six months only about one-third remains. When potatoes are scraped the loss in weight is about 5%; peeling involves a loss of about 20%. Since the concentration of ascorbic acid is lower near the peel, only about 15% of the total is lost with the peelings. Potatoes also contain vitamin B₁ and the members of the B₂ complex.

Effect of Cooking. Cooked starch is more digestible than raw, the grains being gelatinised by wet or dry heat. Dry heat (e.g., baking) converts some of the starch to soluble starch and dextrin. Heating in contact with water causes loss of soluble material, but this is largely prevented if the skins are not removed; cutting potatoes assists extraction. There are certain destructive losses during cooking, the amount depending upon the method.

Baking. Water is the only constituent which is directly lost, but some small destruction of vitamins occurs by the combined action of enzymes and heat.

Frying. On account of the high temperature the destructive losses may be considerable.

Boiling. The effect of boiling is to increase the water content slightly, to lose soluble matter and to destroy vitamins to some extent. The loss of soluble carbohydrates and nitrogenous compounds is not important. Of the minerals, sodium and potassium chlorides show the greatest losses, but iron and magnesium salts may also be reduced considerably. The total losses are small when compared with the daily intake and may be dietetically insignificant. The most important loss is that of the vitamins, particularly ascorbic acid, since cooked vegetables form the major source of vitamin C in the winter months for a large proportion of the population, and root vegetables retain it better than leaf vegetables. After ordinary boiling, about 60% of the vitamin C remains, rather more than half the loss being due to destruction and rather less than half due to solution. Even if the potatoes are unpeeled so that there are no solubility losses, there is the loss due to destruction; but the peel can easily be removed after boiling without the loss of material associated with peeling. About a quarter of the vitamin B₁ may be leached out during boiling. To reduce the losses, the water used should be as little as possible and boiling should be rapid as time increases the loss. The potatoes should be placed directly in boiling water and not in cold water, which is subsequently brought to the boil. The use of soda assists destruction, but salt tends to reduce losses. Retaining cooked potatoes hot or reheating cooled, cooked potatoes produces further losses.

Steaming. There is a loss in weight of about 20% due to evaporation of water. The losses due to destruction are similar to those with boiling but there are negligible extractive losses.

The nutritive value of the potato.—H. Chick, *Chem. & Ind.*, 1940, 735.

The potato as food.—*Nature, Lond.*, i/1941, 103.

Rice for human food consists of the kernel only, and, as commercially separated, is still covered with a brown skin (unpolished rice). This skin, together with the germ, is removed by scouring and polishing (polished rice). To improve further the appearance the grain is sometimes "faced" with talc or other substances.

Polished rice is attractive in appearance, but except as a source of carbohydrates, it is the least valuable of the cereals from the nutritional point of view. Almost the whole of the vitamin B₁ is removed by the polishing. To the natives of many oriental countries, rice is the staple food, and those who consume polished rice suffer from beri-beri, while those who have unpolished rice do not.

Ground Rice is derived from polished rice.

Sago is a starch prepared from the pith of the sago palm, and is of a pale buff colour. The best quality has a slightly reddish tint and readily disperses into a jelly with boiling water.

Tapioca is prepared from the pulp of the roots of Cassava by elutriation with water. After drying in the sun the starch is slightly roasted and dried on warm plates. The product is graded by sifting.

For Vitamin Contents of Cereals see Vitamin Tables, p. 768.

JAM

The Ministry of Agriculture established in 1934 a Standard for jam of superior quality prepared from fresh fruit grown in the United Kingdom and in the **Agricultural Products (Grading and Marking) (Jam) Regulations, 1934**, prescribed a grade designation as follows:—

"Select (Fresh Fruit) Preserve"

"and the quality indicated by such grade designation shall be deemed to be as defined in the First Schedule hereto."

It contains fifteen varieties of preserve, and differs from the Schedule contained in the Regulations of 1933 in that five of the preserves are separated from the remainder on the point of the introduction of other materials.

In common with the other preserves they shall contain no added colouring or flavouring material or preservative, but they may contain an added quantity of any other substance which occurs as a natural constituent of the fruit. This is in order to secure a "satisfactory set," and thus admit of the introduction of pectin, and also acids where a deficiency of these occurs.

These are all single-fruit preserves, and it is laid down that they shall be made from sound and clean fresh fruit and pure sugar, and shall contain no added sweetening material other than sucrose or invert sugar.

This **National Mark Scheme for Jam** was designed to encourage the production of jam of standard quality from home-grown fruit. Large quantities of fruit of varieties grown in this country are imported for manufacture into jam. They come in as whole fruit and pulp, canned or preserved, or as dried fruit (also preserved), and the purchaser is not usually in a position to distinguish between jam made from these and the product of home-grown fruit.

"It is estimated that some 40% of the jam made in this country—other than that made from varieties of fruit which cannot be grown here commercially—is produced from imported fruit or pulp."—Ministry of Agriculture, Marketing Leaflet, No. 36.

Important features of the Scheme are the efforts made to ensure that the jam may be readily identified, and the provision, under Government control, of a guarantee both of the origin of the product, and of the observance of standards of quality officially defined. It includes a system of inspection supported by systematic and regular analyses.

The above standards do not apply in war-time; the present (1943) standards for jam and marmalade are given in S.R. & O. 42/2482, as amended by 43/369, see p. 849.

The need for improvement in the conditions of manufacture and the quality of jam has been felt for some time, and standards were adopted in 1930 by the Food Manufacturers' Federation in consultation with the Society of Public Analysts (*Analyst*, November, 1930).

These cover single-fruit and mixed jams, and prescribe for first and second qualities, to be labelled "**Full Fruit Standard**" and "**Lower Fruit Standard**" respectively, with a guarantee on the label, and each manufacturer is to undertake to conform to the standards before he shall be entitled to use the descriptions.

In the preparation of these jams, of course, fruit other than fresh may be used, the percentage of soluble solids—68.5—is in agreement with that required by the Ministry of Agriculture, but the minimum quantity of fruit required to be used in making 100 lb. of finished jam is lower, ranging, for single-fruit jams, from 30 to 45 lbs., while the Ministry requires 40 to 55 lbs., according to the variety of fruit.

The use of glucose is not prohibited, and added fruit juice and pectin may be present, without declaration in the case of first quality jams. The use of citric, tartaric and malic acids and of "permitted" artificial colouring matter is also allowed without declaration.

Tables are given of the minimum percentages of fruit-content in mixed jams, the minimum of each named fruit being given by the figures in brackets; thus, for first quality mixed jam, whose total minimum in every case is 40%:—

| Fruit content per cent. | | |
|---------------------------|-----|------------|
| Strawberry and gooseberry | ... | 40 (20/20) |
| Gooseberry and strawberry | ... | 40 (30/10) |

the name of the fruit forming the larger content appearing first, where the proportions are not equal.

20% is the minimum fruit content for second quality jams, whether single-fruit or mixed.

In addition to the above there are very varied regulations in force in other countries to some of which British jams may be exported. These regulations are discussed and arranged in comparative tables by C. L. Hinton in *A Summary of Food Laws and Regulations* (Nema Press).

ANALYSIS OF JAM

It will be seen from the standards referred to, and the schedule reproduced above, that an examination of jam includes determination of the total soluble solids, and of the proportion of fruit used in the preparation of the sample.

The latter is a difficult problem the solution of which depends largely on a knowledge of the amounts of insoluble solids, acids and pectin contained in the fruit used, and these vary rather widely in different samples of the same fruit. But by consideration of all three figures a fairly good approximation may be arrived at in the case of single-fruit jams.

A paper on the composition of fruits as used for jam manufacture has been published (*Analyst*, 1931, 35) by T. Macara, Director of Research for the British Association of Research for the Jam and other Trades. In it appear the tabulated results of a large number of analyses of different kinds of fruit carried out during the preceding seven years. Details are given of the methods employed; emphasis is laid on the preliminary preparation and mixing of the sample, and on the importance of analysing a jam in a similar manner to that used for the fruits if the data given for the fruit are to be used in interpreting the results of the jam analysis.

The following directions for the analysis of jam, therefore, conform in outline to the description of the methods employed for the fruit when it is a question of obtaining comparative figures.

Preparation of a 20% Extract

Mix the sample thoroughly in a mortar or small mincer (stones having been removed and their proportion noted). Boil 50 g. moderately with 200 ml. of water for one hour, maintaining the volume during boiling. Cool and make up to 250 ml. Mix well and strain or filter.

Soluble Solids may be obtained from the specific gravity or the refractive index (by immersion refractometer) of the 20% extract, the percentage of solids being calculated by means of the appropriate sugar factors (*Analyst*, 1931, 395).

The soluble solids, of course, are required to conform to the standard of 68.5%, when determined by refractometer reading in the cold (20° adopted), uncorrected for insoluble solids.

They may be determined directly on the sample which has been thoroughly mixed, other types of refractometer being used. A table prepared by Hinton (loc. cit.) gives the percentages of sugar corresponding to the refractive index or butyrefractometer number observed, for use in the absence of a refractometer reading sugar percentages directly.

In the case of the immersion refractometer, which also has an arbitrary scale, the refractive indices may be found by reference to a table in Leach's *Food Inspection and Analysis* (4th Edn., p. 102).

The very small amount of substance used renders it necessary that great care should be taken in mixing the sample if erroneous results are to be avoided.

Sugars. Treat a portion of the extract with lead subacetate, make up to a suitable volume, filter and remove excess of lead from part of the filtrate by means of sodium phosphate. Invert the clean filtrate at 80° with hydrochloric acid, cool, neutralise with sodium hydroxide, and determine the reducing sugar by copper reduction (either gravimetrically or by the volumetric method of Lane and Eynon—see this volume, p. 800).

Acidity. Dilute 50 ml. with several hundred millilitres of water, and titrate with N/10 sodium hydroxide, using phenolphthalein solution as indicator. Deduct any blank obtained by titrating an equal volume of water, and express the result as crystalline citric acid or, in the case of apples, as malic acid.

Pectin (*Spec. Rep. Food Invest. Bd. Lond.*, No. 33, 73); cf. Method of Carré and Haynes, *Biochem. J.*, 1922, 63).

Take sufficient of the *filtered* extract to yield from 0.02 g. to 0.03 g. of calcium pectate; neutralise, and dilute so that after addition of all reagents the total volume measures about 500 ml. Add 100 ml. of N/10 sodium hydroxide and allow to stand at least an hour, preferably overnight. Add 50 ml. of N/1 acetic acid, and after 5 minutes, 50 ml. of M/1 calcium chloride. Allow to stand for one hour, boil for a few minutes and filter through a large fluted filter paper. Wash with boiling water until the filtrate is free from chloride, wash the precipitate back into the beaker, boil and filter again. Repeat unless the filtrate gives no indication of chloride. Filter through a Gooch crucible or tared filter, and dry at 100° to constant weight.

In the foregoing method the pectin is determined as calcium pectate. The following points from pages 27 and 28 of the report with reference to pectin and its related compounds may be of interest here.

- (1) An insoluble pectic compound occurs in unripe fruits and other plant tissues, and is generally described as pectose or protopectin. *Pectose* may be regarded as a pectin-cellulose complex with a constitution analogous to that of the glycosides.

- (2) *Pectin* is a neutral methoxy ester of pectic acid and contains 11.76% of methyl alcohol.

Between pectin and pectic acid are intermediate forms which exhibit increasing acidic properties with a decrease in their content of methyl alcohol.

These intermediate forms are classed together as *pectinic acids* to distinguish them from true pectin.

- (3) *Pectic acid* may be regarded as the basal molecule of pectin, and is a complex galacturonic acid combined with arabinose and galactose.

In pectin the carboxyl groups of the galacturonic acid are replaced by methyl alcohol, and both methyl alcohol groups and carboxyl groups are present in pectinic acids.

- (4) Pectin can be converted quantitatively into calcium pectate, whereby the methoxy groups are removed by hydrolysis and the free carboxyl groups are subsequently replaced by calcium. *Calcium pectate* is a definite chemical compound with an ash content found by experiment to be 7.62% of calcium (Carré and Haynes, 1922).

- (5) The formula of pectin may be provisionally accepted as $C_{20}H_{30}O_{18}$, and the basal molecule, pectic acid, will therefore be $C_{20}H_{28}O_{18}$, with a theoretical calcium content of 7.66%.

Pectin is the only member of the group of pectic compounds which is of commercial importance in the jam and jelly industries.

The pectin is extracted from plant material rich in pectic substances, such as citrus fruits, beets, apples, turnips, etc.

The minced material is either subjected to the action of superheated steam or is heated in water under pressure. Some investigators have recommended extraction of the pectin with dilute acid.

In all cases, however, the underlying principle is the same, viz., the hydrolysis of pectose with the production of pectin.

Pectin for commercial use is prepared either in the form of concentrated extracts, or as a dry powder obtained by precipitating the aqueous extract with alcohol.

The relation of pectin to jelly formation is discussed in the report, and also the legitimate use of tasteless and colourless pectin prepared from fruits, such as apple, to "supplement fruits required for jam making which are naturally deficient in pectin."

Certainly this procedure would seem to be a distinct advance on the older method of introducing foreign ingredients such as gelatin and agar.

Insoluble Matter. Take 10 g. of the well-mixed minced material, dilute with 100 ml. of water and boil for 30 minutes, pour on to a tared filter, return the residue to the beaker and boil with more water. Finally wash on the filter with boiling water and dry in an oven at 105°.

The figures used in calculating the proportion of fruit in jam are those for insoluble solids, acidity and pectin. The *Analyst*, 1931, 39, gives a table of extreme and average figures for various fruits. It is suggested that the composition should first be calculated with the use of the average figures. If the jam falls below the standard, the three minima should be used. If it is still below there is little doubt that it was not made with the standard weight of fruit. A warning is added, however, with regard to the difficulty of ensuring even distribution of the fruit and therefore recommending where possible the examination of more than one jar. Where more than one jar from a boiling is tested it is found that the insoluble solids may vary somewhat widely, but acid and pectin remain much the same, especially the acid.

The acid figure will generally give a fair indication of the composition, except when acid has been added.

Mixed jams present greater difficulties, and call for further methods such as counting and identifying seeds, and for a general microscopical examination, which should be made in every case.

A dissecting microscope is frequently useful for enumerating characteristic elements which do not require high magnification for their detection.

Under the title "The Composition of Fruit," Lampitt and Hughes publish tables of analyses of fresh fruit, covering a period of 2 years (*Analyst*, 1928, 32). Maximum, minimum and average results are given for total solids, total sugars (as invert sugar), pectin (A.O.A.C. method), and insoluble solids. These are of value for reference and comparison, allowing for any variations due to possible differences in the methods of determination.

Hughes and Maunsell have published further tables (*Analyst*, 1934, 231), in which another figure, the non-sugar solids, is included, the authors considering it useful in calculating the amount of fruit in canned fruit, jam, etc.

In the same number, p. 248, Hinton advocates the examination of fruit and jam by lead precipitation for the same purpose.

Examination for Glucose, Saccharin, Tartaric Acid and Preservatives
Jams require examination also for the presence of glucose, saccharin, thickening agents, such as gelatin and agar, for preservatives, colouring matter, etc.

Glucose. A simple polarimetric test for the detection of glucose is given by Judd Lewis (*Analyst*, 1930, 384) who points out that the specific rotation of the inverted extract of all fruit juices is approximately -20° .

With 10% of glucose present a specific rotation of $+1.2^\circ$ was obtained. A positive or reduced negative result therefore indicates glucose.

Saccharin has been found in jam as a sweetening agent. It may be extracted by an ether light petroleum mixture from a solution acidified with dilute sulphuric acid, and, after removal of the volatile solvent, detected by its sweet taste, and by fusing with caustic alkali, acidifying and testing for salicylic acid by weak ferric chloride or iron alum solution. Salicylic acid itself is assumed absent. If not it can be removed by oxidising with alkaline permanganate before the fusion with sodium hydroxide. Benzoic acid, if present, can be removed by sublimation at 100° .

Tartaric Acid. Regulations made under the Agricultural Produce (Grading and Marking) Act, 1928, require "Select Cider, Champagne process" to be free from any acid foreign to apples, and "Select" cider to contain not more than 1 g. per litre of tartaric or citric acids. The absence of tartaric acid can be shown qualitatively; 3 mg. of tartaric acid by the test described gives a distinct precipitate of calcium racemate.—J. King, *Analyst*, 1933, 135.

The method is applicable to food substances such as jellies, jams or other fruit products, but these may require preliminary treatment for the removal of colloidal matter, etc.

Preservatives: Sulphur Dioxide. The only preservative permitted in jams is sulphur dioxide and its compounds which are allowed in ordinary jams to the extent of 40 parts of sulphur dioxide per million, but not, of course, in National Mark preserves. A qualitative test may be made with a simple apparatus devised by Parkes (see illustration: *Analyst*, 1926, 620) consisting of a conical flask fitted with a rubber cork holding a bent two-bulbed thistle funnel which contains a solution of iodine and barium chloride.

The sample, whether jam or other material, is placed in the flask with water, a little porous pot, a few drops of copper acetate solution to retain any sulphide, pieces of marble to replace the air in the flask by carbon dioxide, and finally, just before the cork is inserted, a sufficient quantity of strong hydrochloric acid. When the air is displaced the mixture is gradually brought to the boil, and if

the sample contains sulphur dioxide the first few drops of distillate condensing in the funnel produce turbidity due to the formation of barium sulphate, the iodine solution being more or less decolorised if much sulphur dioxide is evolved.

If the presence of any sulphur dioxide is indicated a quantitative determination must be made. A larger amount of sample is distilled with the same reagents and the distillate collected in a receiving flask containing excess of an iodine solution of approximately decinormal strength. During the greater part of the distillation the condenser tube should dip below the surface of the iodine solution.

When the operation is completed the sulphuric acid formed in the distillate is determined as usual by precipitation with barium chloride, and the sulphur dioxide in the sample calculated from the weight of barium sulphate obtained.

Salicylic and Benzoic Acids may be separated from the sample by shaking an aqueous extract acidified with sulphuric acid with ether, which is washed with a little water, divided into two portions and evaporated in porcelain dishes. The residue in one is tested with weak ferric chloride for the violet colour given by salicylic acid. The slight colour extracted from the jam is usually not enough to interfere with the qualitative test. In a quantitative determination by colorimetric means the jam extract is first treated with a clarifying agent to remove the colour.

The residue in the second dish is examined for crystals of benzoic acid; about 1 ml. of concentrated sulphuric acid and a small crystal of potassium nitrate are added and the dish heated on a boiling water-bath for half an hour. The contents, which should be nearly colourless (slightly yellow if much benzoic acid is present) are diluted with a little water and cautiously made alkaline with ammonia, boiled to decompose any nitrite, cooled and treated with a fresh colourless solution of ammonium sulphide. If benzoic acid is present a red-brown ring or coloured solution of the ammonium salt of meta-diaminobenzoic acid is formed.

Minute traces of salicylic acids may be derived from the fruit composing the jam.

Similarly when the turmeric test for boric acid is applied to the ash of fruit a slight reaction is frequently obtained owing to the presence of a trace occurring naturally.

Canned Fruits

Statutory Rules and Orders (1933; No. 538) made by the Minister of Agriculture and Fisheries prescribe grade designations and grade designation marks for apples, blackberries, cherries, and certain other fruits, produced and canned in England and Wales.

VINEGAR

Vinegar has been described in an old work as that form of acetic acid which is generally preferred for culinary purposes, and which is made by the fermentation of vegetable substances.

This definition recognises the important fact that a true vinegar is produced by a fermentation process, and although the term "vegetable substances" seems rather vague and comprehensive at first, vinegar is actually produced from fermentable matter derived from several sources, and hence we have various descriptive terms such as malt vinegar, wine vinegar, cider vinegar, etc.

When wine, beer and weak spirituous liquids are exposed to the air they become sour owing to the action of a ferment, *Mycoderma aceti*, which brings about oxidation of the alcohol with the production of acetic acid. Various other compounds, including aldehyde, acetic ether, etc., are produced in small quantities at the same time.

Strong wines and spirits are not affected, as the ferment is either killed or its action inhibited. On the other hand, weak

solutions of a pure alcohol do not contain the nitrogenous and other food material required by the organism.

Malt vinegar, sometimes known as British vinegar, is made by brewing a "wort" from malt, i.e., the malt, or mixture of malt and unmalted grain, is crushed and steeped in water at about 60° until by the action of diastase the starch is decomposed with the production of maltose, etc. The process is known as "mashing," and the object is to produce as much fermentable sugar as possible. The cleared extract or wort is treated with yeast to bring about alcoholic fermentation, and the alcoholic "wash" is then transferred to an acetifier, where it is caused to trickle over birch twigs or beech shavings which are impregnated with vinegar to introduce the ferment, meeting with an upward current of air which supplies the necessary oxygen.

A little alcohol is left unconverted to allow for the formation of esters which, with the traces of aldehyde, etc., give the vinegar its characteristic flavour and smell.

The Local Government Board in 1911 described vinegar as a liquid derived wholly from alcoholic and acetous fermentation. A malt vinegar is derived wholly from malted barley or wholly from cereals, the starch of which has been saccharified by the diastase of malt.

This admits of the use of grains other than barley, such as rice and maize, and introduces variations in the composition of vinegars, all entitled to be termed malt vinegar.

Hamill in his Report to the L.G. B. in 1908 refers to the use of acid hydrolysis of maize and rice in preparing a wort, and to the addition of sugars.

Artificial vinegar is any vinegar, or substitute for vinegar, containing, or derived from, any preparation containing any added acetic acid which is not wholly the product of alcoholic and subsequent acetous fermentation.

Vinegar (including all varieties) should contain not less than 4 g. of acetic acid per 100 ml.; arsenic should not exceed 0.0143 g. per 100 ml., and it should not contain any sulphuric or other mineral acid, lead or copper, or any foreign substance or colouring matter except caramel.

With reference to the definition of artificial vinegar, it is pointed out by Cox (*Chemical Analysis of Foods*) that the presence of a minute quantity of mercury is strongly indicative of the use of strong acetic acid to fortify a weak vinegar, much acetic acid being now prepared synthetically from acetylene, using a mercury catalyst. In view of the minute quantity present in such a case, ordinary chemical tests will be useless and recourse must be had to electrolysis with the use of a small gold cathode. If the mercury present is not sufficient to produce a silvery effect on the cathode, it may still be detected by the production of a sublimate of red mercuric iodide. With practice and care 0.02 mg. may be detected. The spectroscope affords an even more sensitive test.

DEFINITIONS OF VINEGAR AND MALT VINEGAR

The following definitions are suggested standards for vinegar and malt vinegar agreed between the Society of Public Analysts and other Analytical Chemists and the Malt Vinegar Brewers' Federation:—

- (i) Vinegar is a product of the alcoholic and acetous fermentation of a saccharine solution without any intermediate distillation, except in the case of spirit vinegar as defined in (iv).
- (ii) Malt vinegar should be derived, without intermediate distillation, wholly from malted barley, with or without the addition of entire cereal grain, malted or otherwise, the starch of which has been saccharified by the diastase of malt.
- (iii) When vinegar is demanded, a purchaser should be supplied with malt vinegar, unless due notification is given to the purchaser of the article supplied.
- (iv) The name "Vinegar" may be applied to other products which comply with the definition of vinegar (No. (i) above), provided a prefix is used to denote the origin of the product; thus, "Distilled Vinegar," "Spirit Vinegar."

Distilled vinegar is the product of the distillation of vinegar as defined in No. (i) above, and its source should be denoted; such as, distilled malt vinegar.

Spirit vinegar is the product of the acetous fermentation of a distilled alcoholic fluid.

- (v) "Imitation" or "Artificial" vinegar should in every case be sold specifically marked "Imitation" or "Artificial" vinegar. It is any substitute for vinegar containing acetic acid which is not wholly the product of alcoholic and subsequent acetous fermentation, and shall not contain any acid other than acetic acid.
- (vi) All vinegars and imitation artificial vinegars shall contain not less than 4% w/v of acetic acid. They shall not contain any acid other than acetic acid or those acids produced by normal fermentative processes.
- (vii) Caramel may be used as a colouring matter in all vinegar and in "Imitation" or "Artificial" Vinegars.

By a London magistrate's ruling, confirmed at London Sessions on appeal, "vinegar" and "table vinegar" must be prepared by acetous fermentation.—*Analyst*, 1937, 384, 605.

Wine vinegar is prepared on the Continent by acetous fermentation of inferior wines, the best being made from white wine in France, chiefly at Orleans.

Cider vinegar is principally an American product. It contains a considerable amount of malic acid.

Concentrated vinegar or vinegar essence is usually prepared by adding acetic acid to brewed vinegar until 25 to 30% of acetic acid is present (Hamill).

It seems probable that some of these concentrated preparations contain no brewed vinegar, but consist of acetic acid and caramel suitably flavoured. When diluted, they produce a cheap artificial vinegar. In fact, they serve in poor districts for the extemporaneous preparation of vinegar by the retailer, who is frequently too liberal with the water, and so supplies a vinegar containing less than 4% of acetic acid. In a particular instance, 1 gallon of the strong essence, when diluted, yields 12 gallons of the artificial vinegar. Such products are sometimes known by the term "wood vinegar."

The Food and Drug Administration of the U.S. Dept. of Agriculture defined vinegar, cider vinegar, or apple vinegar, as the product made by the alcoholic and subsequent acetous fermentations of the juice of apples. *Wine vinegar*, grape vinegar: from the juice of grapes. *Malt vinegar* from an infusion of barley malt or cereals whose starch has been converted by malt. *Sugar vinegar* from sugar syrup, molasses, or refiners' syrup. *Glucose vinegar* from a solution of glucose is dextrorotatory. *Spirit vinegar, distilled vinegar, grain vinegar*, from acetous fermentation of dilute distilled alcohol. All vinegars contain in 100 ml. (20°) not less than 4 g. of acetic acid.—*S.R.A.*, *F.D.* No. 2, *Rev.* 5, Nov. 1936.

Analysis of Vinegar

A general analysis of vinegar will include a determination of the specific gravity, about 1.019 in the case of a good malt vinegar, usually much less, but variable in artificial vinegar; the total solids, in malt vinegar about 2 to 2.5%,

may be only 0.2% or 0.3% in artificial vinegar, the ash having figures correspondingly small and giving practically no reaction for phosphates which, of course are present in malt vinegar.

The ash of vinegar should be tested for reaction and should be alkaline to litmus.

The acidity may be determined by titration with N/2 sodium hydroxide solution using phenolphthalein as indicator. 5 or 10 ml. of vinegar are well diluted with water in a large porcelain dish. The colour change is seen more clearly in the white dish, and the end-point may be made still clearer by using a second dish with the same amounts of vinegar and water for comparison. Each millilitre of N/2 NaOH is equivalent to 0.03 g. of acetic acid.

Further analysis should include determinations of **nitrogen** and **phosphoric acid**, and tests for free sulphuric acid, ferrocyanides, arsenic and poisonous metals.

Ferrocyanides are sometimes used to clarify vinegar. They may be tested for with a weak solution of ferric chloride.

Tests should also be made for sulphur dioxide (see page 831) and other preservatives.

Free mineral acid is improbable if the ash is alkaline to litmus. A further test applied to a little of the vinegar is based on the fact that, in the presence of alcohol, acetic acid in all concentrations gives a yellow colour with methyl orange, whereas a strong acid gives a pink in all concentrations down to 0.005% (Prideaux).

The presence of a small amount of sulphuric acid was at one time permitted.

Free sulphuric acid may be detected and estimated by *Hehner's method*. Evaporate 50 ml. of sample in a platinum dish with 25 ml. of N/10 NaOH, and ignite at a low temperature. Add 25 ml. of N/10 acid, boil, filter and wash with hot water. Titrate the filtrate and washings with N/10 NaOH using phenolphthalein as indicator. Each millilitre of N/10 NaOH required is equivalent to 0.0049 g. of H_2SO_4 .

Alternative method. To a measured quantity of sample add a measured excess of standard alkali, evaporate to dryness, incinerate, and titrate the ash with standard acid, using methyl orange as indicator. The difference between the number of millilitres of alkali first added and the number of millilitres of acid needed to titrate the ash represents the free mineral acid present.

Differentiation of Malt, Distilled Malt, Spirit and Artificial Vinegars. (Edwards and Nanji, *Analyst*, 1938, 410). The authors describe precise methods for determining the oxidation value, iodine value and ester value of the volatile reducing substances separated by distillation, and discuss their significance for different types of vinegar.

OXIDATION VALUE. The number of millilitres of 0.01N potassium permanganate used up by 100 ml. of vinegar in 30 minutes under the standard conditions. Mix 60 ml. of the sample with 15 ml. of water in a 400 ml. distilling flask, add a little pumice and distil the mixture slowly until exactly 60 ml. are collected. Measure 25 ml. of the distillate into a glass-stoppered bottle of about 200 ml. capacity, add 10 ml. of dilute sulphuric acid, 1 in 4 by volume, followed by 10 ml. of 0.1N potassium permanganate solution, accurately measured. Allow the mixture to remain at a temperature of about 18° for exactly 30 minutes; add 5 ml. of 10% potassium iodide solution and titrate the liberated iodine with 0.02N thiosulphate, adding starch solution as indicator at the end. Carry out a blank determination at the same time, using 25 ml. of distilled water in place of the vinegar distillate. Let the volume of 0.02N thiosulphate used in the blank and test titrations be A and B. The oxidation value is $8(A-B)$.

IODINE VALUE. The number of millilitres of 0.01N iodine absorbed by 100 ml. of vinegar under the standard conditions. Measure 25 ml. of the distillate into a glass-stoppered bottle of about 200 ml. capacity and just neutralise with 10N potassium hydroxide to litmus paper. Add 10 ml. of N potassium hydroxide followed by 10 ml. of 0.1N iodine solution accurately measured. Allow the reaction mixture to stand at room temperature in the dark for 15 minutes; add 10 ml. of dilute sulphuric acid, 1 in 4 by volume, and titrate the liberated iodine with 0.02N thiosulphate solution. Carry out a blank test in an exactly similar manner, substituting for the vinegar 25 ml. of distilled water. Let the volume of 0.02N thiosulphate used in the blank and test titrations be A and B. The iodine value is $8(A-B)$.

ESTER VALUE. The number of millilitres of 0.01N potassium hydroxide required to saponify the esters contained in 100 ml. of vinegar under the standard conditions. Measure 100 ml. of sample into a 400 ml. distilling flask, add a little pumice and distil slowly until 30 ml. are collected. Make just alkaline to phenolphthalein with N potassium hydroxide. Add 0.02N hydrochloric acid, drop by drop, from a burette until the colour is just discharged; add 10 ml. of 0.01N potassium hydroxide, accurately measured, and saponify by heating the mixture under a reflux on a boiling water-bath for 2 hours. Cool and titrate the excess alkali with 0.02N hydrochloric acid. For a blank test, take about 30 ml. of water and 10 ml. of 0.1N potassium hydroxide solution, heat under a reflux in a similar manner and titrate with 0.02N hydrochloric acid, using the same quantity of phenolphthalein as in the actual test. Let the volumes of 0.02N hydrochloric acid used in the blank titration and in the test be A and B. The ester value is $2(A-B)$.

The figures for malt vinegars and distilled malt vinegars are much higher than for spirit vinegars or artificial vinegars. The oxidation values for spirit vinegars are much higher than for artificial vinegars. The iodine values divide the vinegars (other than malt) into three classes, the spirit vinegars having the intermediate figures, the artificial the higher and lower figures. The ester values are interpreted with the oxidation values and iodine values. It should be pointed out that these new values should be used in conjunction with the older determinations.

Whitmarsh (*Analyst*, 1942, 188) considers that the distinctive values for spirit vinegar are due to the alcohol content, those for malt vinegar largely, if not solely, to the presence of alcohol and acetyl methyl carbinol. (See also Manley, *Analyst*, 1940, 594, for further analytical data and conclusions.)

The following method will detect small quantities of added mineral acid (e.g., 0.1 to 0.2%). The vinegar is shaken with animal charcoal, allowed to stand for one hour and filtered. The reaction of the filtrate is found by means of a Hellige Comparator, thymol blue being used as indicator. The criterion with malt vinegars is a pH of the filtrate of 2.9 as against 2.5 for 4% acetic acid or artificial vinegar).—D. C. Jenkins, *Analyst*, 1941, 328.

Pickles and Sauces, made from fruit or vegetables, are permitted to contain benzoic acid to the extent of 250 parts per million.

The benzoic acid may be determined by the method used for ketchup in *Methods of Analysis (A.O.A.C.)*. A weighed quantity is saturated with sodium chloride, made slightly alkaline with 10% soda and made up to volume with salt solution, shaken frequently during 2 hours, squeezed through muslin and filtered. A portion of the filtrate is acidified with HCl, extracted with chloroform four times, the combined chloroform extracts evaporated in a current of dry air, and dried in a desiccator over sulphuric acid until no odour of acetic acid is detected; the residue is dissolved in neutral alcohol and titrated with N/20 NaOH using phenolphthalein.

An alternative method due to Monier-Williams (Report to Ministry of Health, 1927), consists in saturating the sample with sodium chloride, acidifying with phosphoric acid, and distilling in steam into a dish containing N/1 alkali. The alkaline distillate is evaporated, purified by treatment with permanganate, saturated with NaCl, and extracted with equal volumes of ether and light petroleum (b.p. 30° to 50°). The solvent is evaporated in a test-tube and the benzoic acid mixed with sand and sublimed into the upper part of the tube, which is cut off, dried in a desiccator, weighed, the benzoic acid removed, and the tube re-weighed.

FOOD PRESERVATIVES

The Minister of Health issued Regulations based on recommendations of the Departmental Committee on the Use of Preservatives and Colouring Matter in Food, and providing for the prohibition of the importation and sale of articles of food to which preservatives and other specified substances have been added. These Regulations in general came into operation on January 1st, 1927.

The Regulations prohibit the use in foodstuffs and drinks of preservatives except those mentioned below, and then on condition that they do not contain a larger proportion than is specified, and are properly labelled.

"Preservative" includes any substance which is capable of inhibiting, retarding or arresting the process of fermentation, acidification or other decomposition of food or of masking any of the evidences of putrefaction.

The term does not include Salt, Saltpetre, Sugars, Vinegar, Acetic or Lactic Acid, Alcohol, or potable Spirits, Spices, Herbs, Hop Extract, Essential Oils used for flavouring, Glycerin or any substance added by the process of curing known as smoking.

PERMITTED PRESERVATIVES

| Food | Preservative | Parts per million | |
|--|-----------------|-------------------|------------------------|
| (1) Sausage and sausage meat containing raw meat, cereals and condiments | SO ₂ | 450 = | 3.15 grains per lb. |
| (2) Fruit and fruit pulp not dried:— | | | |
| (a) Cherries | SO ₂ | 3000 = | 21.0 " " " |
| (b) Strawberries and raspberries | SO ₂ | 2000 = | 14.0 " " " |
| (c) Other fruit | SO ₂ | 1500 = | 10.5 " " " |
| (3) Dried fruit:— | | | |
| (a) Apricots, peaches, nectarines, apples and pears | SO ₂ | 2000 = | 14.0 " " " |
| (b) Raisins and sultanas | SO ₂ | 750 = | 5.25 " " " |
| (4) Unfermented grape juice and non-alcoholic wine made from such grape juice if labelled in accordance with the rules contained in the second schedule to these Regulations | Ac. Benz. | 2000 = | 140.0 grains per gall. |
| (5) Other non-alcoholic wines, cordials and fruit juices, sweetened or unsweetened | SO ₂ | 350 = | 24.5 " " " |
| | or Ac. Benz. | 600 = | 42.0 " " " |
| (6) Jam (including marmalade and fruit jelly prepared in the way in which jam is prepared) | SO ₂ | 40 = | 0.28 " " lb. |
| (7) Crystallised glacé or cured fruit, including candied peel | SO ₂ | 100 = | 0.7 " " " |
| (7a) Fruit and fruit pulp not otherwise specified in this schedule | SO ₂ | 350 = | 2.45 " " " |
| (8) Sugar (including solid glucose) and cane syrups | SO ₂ | 70 = | 0.49 " " " |
| (8a) Cornflour (maize starch) and other prepared starches | SO ₂ | 100 = | 0.7 " " " |
| (9) Corn syrup (liquid glucose) | SO ₂ | 450 = | 3.15 " " " |
| (10) Gelatin | SO ₂ | 1000 = | 7.0 " " " |
| (11) Beer | SO ₂ | 70 = | 4.9 " " gall. |
| (12) Cider | SO ₂ | 200 = | 14.0 " " " |
| (13) Alcoholic wines | SO ₂ | 450 = | 31.5 " " " |
| (14) Sweetened mineral waters | SO ₂ | 70 = | 4.9 " " " |
| | or Ac. Benz. | 120 = | 8.4 " " " |
| (15) Brewed ginger beer | Ac. Benz. | 120 = | 8.4 " " " |
| (16) Coffee extract | Ac. Benz. | 450 = | 31.5 " " " |
| (17) Pickles and sauces, made from fruit or vegetables | Ac. Benz. | 250 = | 1.75 grains per lb. |

Sulphur dioxide includes sulphites, and benzoic acid includes benzoates, calculated respectively in terms of sulphur dioxide and benzoic acid.

LABELLING. Special labelling applies to *sausages, sausage meat, coffee extract, pickles and sauces* and (where proportion of benzoic acid exceeds 600 parts per million) *grape juice and wine*. These must bear a label stating the contents are preserved, e.g., "These sausages contain preservative," and in the case of grape juice and wine where it applies "and is not intended for use as a beverage."

The retailer must exhibit a notice in a conspicuous place to the effect that the goods in question contain preservative.

(See also *Alterations in the Composition of Food due to War*, p. 848.)

Departmental Committee's Report.

Preservatives may be used to mask unsoundness, or careless methods of production, storage and distribution. A dose of **boric acid**, the most commonly used preservative, is not completely excreted from the system for 5 days, and the tissues are never free—the Committee considered its prohibition justified. **Sodium sulphite**, in amounts employed in foods, has no specific toxic action, but is not harmless, as sulphur dioxide liberated may cause dyspeptic symptoms. The putrefactive odour of decaying meat is removed by treatment with sodium sulphite, and the red, fresh appearance restored. Prof. A. J. Clark, representing the B.M.A., considered that **formaldehyde** and **fluorides** should be prohibited, as they are definitely toxic, as also **borax** preparations, on account of cumulative action, and **salicylic acid** and **salicylates**, on account of powerful physiological action. Preservatives are used in a haphazard way, or even in ignorance, and often no effort is made to do without them. The Committee saw no reason why the sale of cream should not be conducted on the same lines as milk, i.e. without preservatives. They recommended that after two years of grace preservatives in butter and margarine should be prohibited. Addition of preservatives to sausages is undesirable, and should be regarded as a concession to trade necessities, which should eventually be dispensed with. Use of preservatives for packing and dusting of hams is unnecessary. Recommendation that use of preservatives in liquid eggs should be prohibited—freezing an alternative. Preservatives should be unnecessary in alcoholic wines of ordinary strength, but where required sulphur dioxide should be permitted in amounts not exceeding 3 grains per pint. The Committee finally recommended that preservatives be prohibited in all articles of food or drink, offered or exposed for sale, whether manufactured in this country or imported, apart from certain exceptions which are given *antea*.

The method of estimating preservatives to be prescribed by the Ministry of Health. Sale of food preservatives should be illegal unless they bear descriptions indicating composition and strength, and are free from impurities, containing not more than 1/100 gr. arsenic or 1/7 gr. lead per lb.—*Brit. med. J.*, ii/1924, 290, 828, 829. See also *Brit. med. J.*, ii/1925, 349; i/1927, 70.

DYES USED IN COLOURING FOODS

The following aniline dyes have been found by various authorities to be harmless for colouring foods. Many were given in a list issued by the National Confectioners' Association of the United States in 1899, and to these have been added other colours, specially indicated, which are permitted by the Governments of Canada, the United States and certain Australian States. The number placed before each colour is that of the **COLOUR INDEX OF THE SOCIETY OF DYERS AND COLOURISTS** (1924), which is the standard reference book of the English-speaking countries.

*The dyes marked with a * are stated in the Colour Index to be actually used for colouring edibles.*

The use of these dyes is not prohibited by the Public Health (Preservatives, etc., in Food) Regulations, 1926-7. These Regulations render the use of the following colouring matters illegal:—

1. Compounds of antimony, arsenic, cadmium, chromium, copper, mercury, lead and zinc.

2. Gamboge.

3. Picric acid (7), *syn.* carbazotic acid; victoria yellow (8), *syn.* saffron substitute and dinitrocresol; manchester yellow (9), *syn.* naphthol yellow and martius yellow; aurantia (12), *syn.* imperial yellow; aurine (724), *syn.* rosolic acid and yellow coralline.

The Canadian regulations forbid the use of aniline dyes containing more than 10 parts per million of arsenic, As_2O_3 , or heavy metals (iron excepted), and the dyes must not be used in quantities exceeding 2 grains per lb. (1 in 3500).

Blue

- 689 **Gentian Blue 6B**, *syn.* SPIRIT BLUE, ANILINE BLUE.
The hydrochloride, sulphate or acetate of phenylated *p*-rosaniline and rosaniline. Insoluble in water. The acetate is readily soluble in alcohol, the sulphate and hydrochloride sparingly soluble.
- 861 **Coupler's Blue**, *syn.* WATER-SOLUBLE INDULINE.
Sodium salts of sulphonated mixtures of amino-diphenyl-diamino-triphenyl-triamine and tetra-phenyl-tetramino-phenyl-diphenazonium-chloride.
Soluble in alcohol and water.
- 1180 **Indigo Carmine** (Aus., Can., U.S.A.).
Sodium salt of indigotin-5 : 5'-disulphonic acid.
Soluble in water, sparingly soluble in alcohol.

Green

- 670* **Light Green S.F. Yellowish** (Aus., Can., U.S.A.).
Sodium salt of dibenzyl-diethyl-diamino-triphenylcarbinol tri-sulphonic acid anhydride.
Soluble in water, almost insoluble in alcohol.
Picric acid gives no precipitate (distinction from green basic dyes).

Brown

- 331* **Bismark Brown G** (Aus.).
Hydrochloride of benzene-*m*-diazo-bis-*m*-phenylenediamine.
Soluble in water and alcohol.
- 480 **Chrysamine R**.
Sodium salt of ditolyl-diazo-bis-salicylic acid. Water-soluble.

Orange

- 26 **Crocein Orange**.
Sodium salt of benzene-azo- β -naphthol-6-sulphonic acid.
Slightly soluble in water, moderately soluble in alcohol.
- 150* **Tropaeoline 000**.
No. 1, or Orange 1 (Aus., Can., U.S.A.). Sodium salt
p-sulphobenzene-azo- α -naphthol.
Soluble in water and alcohol.

Red

- 46 **Archil Substitute**.
p-Nitrobenzene-azo- α -naphthylamine-4-sulphonic acid.
Aqueous solution reddish-brown.
- 79* **Ponceau 2R**, *syn.* SCARLET R.
Sodium salt of *m*-xylene-azo- β -naphthol-3 : 6-disulphonic acid.
Yellowish-red solution in water, insoluble in alcohol.
- 80* **Ponceau 3R and 4R**, *syn.* CUMIDINE RED (Aus., Can., U.S.A.).
Sodium salt of cumene-azo- β -naphthol-3 : 6-disulphonic acid.
Ponceau 3R is made from crude cumidine, and Ponceau 4R from pseudo-cumidine.
Water—cherry-red solution; slightly soluble in alcohol.
- 88* **Bordeaux B**, *syn.* ACID BORDEAUX.
Sodium salt of α -naphthalene-azo- β -naphthol-3 : 6-disulphonic acid.
Soluble in water, moderately soluble in alcohol.
- 179 **Carmoisine**, *syn.* AZORUBINE.
Sodium salt of 4-sulpho- α -naphthalene-azo- α -naphthol-4-sulphonic acid. Soluble in water.
- 182 **Fast Red E**.
Sodium salt of 4-sulpho- α -naphthalene-azo- β -naphthol-6-sulphonic acid. Soluble in water and moderately soluble in alcohol.
- 184* **Amaranth** (Aus., Can., U.S.A.).
Sodium salt of 4-sulpho- α -naphthalene-azo- β -naphthol-3 : 6-disulphonic acid. Soluble in water, sparingly soluble in alcohol.
- 370 **Congo Red**.
Sodium salt of diphenyl-diazo-bis- α -naphthylamine-4-sulphonic acid. Soluble in water.

- 677* **Magenta**, *syn.* FUCHSINE, ROSEINE (Aus.).
Mixtures of *p*-rosaniline and rosaniline hydrochlorides.
Soluble in hot water, and in alcohol. Soluble in amyl alcohol
(useful for detection in wine).
- 692 **Acid Magenta**, *syn.* ACID FUCHSINE.
Mixture of salts of di- and trisulphonic acids of *p*-rosaniline and
rosaniline. Soluble in water, almost insoluble in alcohol.
- 768 **Eosin**.
Sodium or potassium salt of tetrabromofluorescein.
Soluble in water and alcohol.
- 773* **Erythrosine** (Aus., Can., U.S.A.).
Sodium or potassium salt of tetraiodofluorescein
- 774 **Phloxin**.
Potassium salt of tetrabromodichlorofluorescein.
Aqueous solution fluorescent.
- 777 **Rose Bengale**.
Potassium or sodium salt of tetraiododichlorofluorescein.
Aqueous solution not fluorescent.

Violet

- 279 **Wool Black**.
Sodium salt of *p*-sulpho-benzene-azo-*o*-sulphobenzene-azo-*p*-tolyl-
β-naphthylamine. Soluble in water.
- 315 **Naphthol Black B**.
Sodium salt of 6 : 8-disulpho- β-naphthalene-azo-α-naphthalene-
azo- β-naphthol-3 : 6-disulphonic acid. Soluble in water.
- 463 **Azoblu**.
Sodium salt of ditolyl-diazo-bis-α-naphthol-4-sulphonic acid.
Soluble in water.
- 680 **Methyl Violet**.
Mixtures of hydrochlorides of higher methylated *p*-rosanilines.
Soluble in water and alcohol.
- 846 **Mauveine**.
Mainly amino-phenylamino-*p*-tolyl-ditolazonium sulphate.
Insoluble in cold water, soluble in alcohol.

Yellow
(Water soluble.)

- 10* **Naphthol Yellow S**. (Aus., Can., U.S.A.).
Potassium or sodium salt of 2 : 4-dinitro-α-naphthol-7-sulphonic acid.
- 16* **Acid Yellow**.
Sodium salt of aminoazobenzene-di-(and mono-)sulphonic acid.
The aqueous solution has a neutral action, mineral acids change the
colour to a bright red, yellow being restored by the addition of alkali.
Used for colouring milk (1 in 200,000), egg powders—custard
prepared for table contains about 1 in 40,000.
- 364 **Brilliant Yellow**, *syn.* PAPER YELLOW.
Sodium salt of 2 : 2'-disulphostilbene-4 : 4'-diazo-bis-phenol.
- 640* **Tartrazine** (Can., U.S.A.).
Sodium salt of 4-*p*-sulphobenzene-azo-1-*p*-sulphophenyl-5-hydroxy-
pyrazol-3-carboxylic acid.
A yellow powder almost unaffected in colour by acids or alkalis.
When tartrazine is reduced sulphanilic acid is formed. Used for
lemonade, etc., a common proportion being 1 in 500,000.

Yellow
(Oil soluble.)

- 15* **Aminoazobenzene**.
Aminoazobenzene hydrochloride (for fats and cheese).
- 17* **Aminoazotoluol**.
Aminoazotoluene or HCl salt (for fats, wax and margarine).
- 19* **Oil Yellow**.
Dimethylaminazobenzene or benzene-azo-dimethyl-aniline (for
oils).

22* Oil Yellow A.B. (U.S.A.).

Benzene-azo- β -naphthylamine (for oils and fats).

61* Oil Yellow O.B. (U.S.A.).

o-Toluene-azo- β -naphthylamine (for oils and fats).

Other harmless colouring agents are madder, logwood, annatto, turmeric, marigold, chrysophanic acid and saffron. Naphthol green, metanil yellow, bismark brown, and methylene blue are stated to be more or less poisonous. Certain dyes, rosaniline for example, are liable to contain arsenic, such as by the use of arsenic acid as oxidising agent, or from the use of crude oil of vitriol containing arsenic.—Kenwood.

Two cases of dermatitis due to the handling of oranges dyed with yellow O.B. dye, which is the permitted food dye used for colouring fruit. Patch tests showed that the dye did not penetrate even to the inside of the peel. While the dyes used on citrus fruits may definitely irritate some skins they are not general irritants, because the majority of individuals do not react to them, but hypersensitivity may be acquired by constant and repeated exposure.—E. F. Traub, R. E. Gordon, and L. S. Van Dyke, *J. Amer. med. Ass.*, i/1937, 872.

Colours in food.—*Nature*, Lond., i/1942, 537.

Of the synthetic aniline dyes relatively few are considered harmless in other countries.—*Final Report of the Food Preservative Committee*, 1924, v. ante.

Annatto Substitute

Is a mixture of acid brown No. 1 (10 parts) and acidyellow (8 parts). *Acid brown* is the sodium salt of para-sulpho-benzene-azo-metatolulylene-diamine (4) (SO_3Na) $\text{C}_6\text{H}_4\text{N}:\text{NC}_6\text{H}_3(\text{CH}_3)(\text{NH}_2)_2(1,5,2,4)$. A dark brown powder with occasional yellow specks dissolving easily in water. The solution has a neutral reaction and is of a dark red colour, becoming yellow when greatly diluted. Mineral acids change the solution to a bright red. Alkalis return original colour.

Used for the same purposes as the vegetable colour (has approximately 25 times the tinctorial power of the commercial extracts of the fruit, of which 1 tablespoonful is added to 30 lb. cheese, i.e., 1 part in 960) for tinting milk, butter, cheese (1 in 24,000), haddocks, etc.

Annatto is obtained from the seeds of *Bixa Orellana*.

Annatto Extract.—Bixin, related to *m*-xylene, is the essential colouring matter. The extract is usually strongly alkaline.

For a bibliography on *heavy metals in food* and biological material compiled by T. H. Pope at the request of the Publications Committee of the Society of Public Analysts, see (I Copper), *Analyst*, 1932, 709; (II Lead), 1932, 775; (III Zinc), 1933, 30; (IV Manganese), 1933, 91; (V Mercury), 1933, 280; (VI Cobalt), VII Nickel, VIII Chromium, 1933, 340; (IX Tin), 1933, 398; (X Bismuth), *Analyst*, 1933, 607; (XI Antimony, XII Cadmium, XIII Thallium) *Analyst*, 1934, 109. (These have now been published in one compilation for the years 1921 to 1933 inclusive, but the individual references are given here for convenience).

A supplementary series has since been published as follows: (I Cobalt), *Analyst*, 1940, 513; (II Nickel), 1940, 603; (III Manganese), 1941, 196; (IV Zinc), 1941, 452; (V Copper), 1942, 293, 324.

FOOD SUBSTITUTES

The Ministry of Food defines a food substitute as any preparation or product offered or purporting to be capable of being used as a substitute for any food. The term "food substitute" includes two very different types of articles and it is better to define each type separately. A food substitute must itself be a food which adequately replaces the food which it purports to substitute either in composition or in function. For example, vitaminised margarine is a good substitute for butter; its composition is similar and its food value practically identical. Baking powder is a good

substitute for yeast in baking though its composition is entirely different. These two types of substitutes should not be confused, and in particular, substitutes of the second type should not be held out as substitutes of the first type. For example, egg-substitute powder which is usually only coloured baking powder replaces eggs for baking purposes; it has none of the nutritive value of fresh eggs, and preparations which are labelled or advertised as equal to so many eggs or in other ways mislead the purchaser, clearly contravene Section 6 of the Food and Drugs Act. Many largely advertised brands of lemonade crystals or powder are prepared with tartaric acid, and liquid preparations of lemonade with a mixture of citric and phosphoric acids. While such articles may be looked upon as adulterated, it might be difficult to obtain convictions as lemonade may be defined as a lemon-flavoured beverage. But if such preparations are offered as lemon substitutes equivalent to so many lemons the substitution of citric acid by tartaric or phosphoric acid constitutes an offence.

It was perhaps inevitable that as stocks of the commoner foods became scarcer and rationing was introduced, substitutes should make their appearance. At first confined to sugar substitutes the number and nature grew rapidly: sweetened custard powder, blancmange powder, self-raising powder, etc., egg substitutes, milk substitutes, substitutes for citrus fruits, and onion substitutes were offered to the public in bewildering variety, accompanied by most extravagant claims as to their nature and value, and packed in highly coloured and decorative containers. Early in 1941 a number of these preparations were adversely reported upon by public analysts and prosecutions followed, usually under Section 6 of the Food and Drugs Act. Defendants were ably represented by Counsel and magistrates were often loath to convict or imposed only nominal fines which were no deterrent to the makers of such enormous profits. Public attention was eventually drawn to the situation by some of the more widely circulated newspapers and by questions in Parliament, and the Ministry of Food issued the Food Substitutes (Control) Order, S.R. & O. 1941, No. 1606, making it an offence to make any food substitute from that date except in accordance with the terms of a licence granted by the Ministry. In spite of the lucrative nature of the trade, most of the makers of food substitutes omitted to apply for a licence. Before granting licences to applicants the Ministry subjected the preparations, labels and prices to careful consideration; coined words or pictures which suggested the food substituted and all extravagant claims were deleted, and in some cases drastic reduction in price was enforced. The Order has put a stop to the exploitation of the public by the makers of worthless articles and, although there are substances of little value still on the market, no prosecutions by the Food and Drugs authorities have been instituted in respect of any product labelled in accordance with the terms of the relevant licence. It is open to the Food and Drugs authorities to institute prosecutions for articles deemed

unsatisfactory, but it is believed that convictions would be difficult to obtain in spite of the fact that the Order specifically provides that the licence shall not operate to relieve the holder from the requirements of any other Act, Order, etc. The composition of these preparations is disclosed to the Ministry, but is not available to the public analyst, so that only the Ministry is in a position to ascertain whether the terms and conditions attached to the licences are being adhered to.

When an abundance of more varied food becomes available few of the hundreds of food substitutes are likely to survive. The same applies to a large number of other food preparations which do not come under the Substitutes Order.

Sugar Substitutes. Saccharin tablets sold as tea-sweetening tablets, each equivalent to one lump of sugar, contained as little as 10 gr. of sugar equivalent: saccharin solutions sold as equivalent to so many pounds of sugar or with a claim that one drop was equivalent to one lump or one teaspoonful of sugar overstated the strength by several times. Sweetening powders consisted of saccharin mixed with glucose or sometimes chalk.

Fruit Juice Substitutes. Most of these purported to be lemon or orange substitutes equal to the juice of so many lemons or oranges and often vitamin C was stated to have been added. Only preparations containing vitamin C were allowed to be described as "Lemon (or Orange) Substitutes"; other preparations are required to be described as "Flavourings." In consequence of the Order many fruit juice substitutes have now had to have their names changed to "Flavourings."

Milk Substitutes. Milk substitutes, like egg substitute powder, have a place in cooking, but have practically none of the virtues of milk as a food. Some of the preparations offered had been sold to bakers for many years for adding to flour: when sold to the public the price increased tenfold. Licences for milk substitutes were only granted in respect of products containing a substantial proportion of soya and even then it was a condition of the licence that the product was not sold to the public. Flour and salt were the usual components, usually with a little gelatin and sometimes with the addition of rice flour or fine oatmeal.

Egg Substitutes. These are of two types. The type which is most familiar to the general public consists simply of coloured baking powder, and has been on the market for many years. The second type consists of gums and other substances; these do not depend on the liberation of carbon dioxide for their action. For use these products require to be mixed with water and vigorously "whipped" so as to form a foam by which air is included in the "mix," thus replacing the carbon dioxide liberated by the effervescing type of powder on moistening. Products of this type have been used extensively in the bakery trade for many years, but were unknown to the public before the war. While their use in the bakery trade may have given satisfactory results, their use in domestic cookery, especially by those who are accustomed to the coloured baking powder type of powder, is less likely to be satisfactory unless the necessity for thorough whipping is appreciated. Manufacturers of the first type of egg substitute powder have agreed at the request of the Ministry of Food to cease using that name and these products will in future be known as "golden raising powder." It would appear that this change of name will in general remove these products from the ambit of the Substitutes Order, but they will be subject to control under the Manufactured and Pre-Packed Foods (Control) Order (*vide infra*).

Onion Substitutes. Oil of garlic or synthetic essences mixed with flour in solid preparations or with isopropyl alcohol in liquids were the most usual substitutes. The odour of garlic may be acceptable to those members of the public whose recollection of onions is now vague, but a solution of asafetida oil in isopropyl alcohol is scarcely a satisfactory substitute.

Other Substitutes. Synthetic cream, an emulsion of neutral fats used for filling cakes and pastry has been sold to replace cream. Custard powder

substitutes and blancmange substitutes are made by replacing the starch (cornflour, rice or arrowroot) by wheat flour.

A paper on food substitutes—egg, milk, meat, fruit juice, sugar and onion substitutes.—E. Voelcker, *Chem. & Ind.*, 1942, 17, 192. See also *Analyst*, 1942, 139.

FOOD AND DRUGS ACT, 1938

The Food and Drugs Act, 1938, is primarily a consolidating Act, no fewer than 250 provisions from 36 Acts of Parliament, dating from 1541, being repealed and replaced by a single measure of 103 sections. The Food and Drugs (Adulteration) Act, 1928, was itself a consolidating Act, replacing a principal Act almost 30 years old and incorporating special legislation dealing with milk and dairies, butter and margarine. The new Act is the outcome of the recommendations of the Local Government and Public Health Consolidation Committee appointed to consider enactments dealing with (1) local authorities and local government, and (2) matters relating to the public health, to regroup these enactments and suggest amendments of the existing law, to facilitate consolidation and secure simplicity, uniformity and conciseness. Thus the chief merit of the Act is that it provides a base upon which to build new legislation as the public interest requires it. The modern tendency of legislating by regulations is extended, and powers are given to the local authorities to make byelaws, and to the Minister of Health and the Minister of Agriculture and Fisheries to make regulations.

Byelaws may be made by local authorities:

(15) with respect to the handling, wrapping and delivery, and the sale or exposure for sale in the open air of food for human consumption;

(56) for the control of markets;

(58) for the management of slaughter-houses and knackers' yards; and byelaws must be made for:

(60) the management of public slaughter-houses.

The Minister of Health may make regulations for:

(8) prevention of danger to health from the importation, preparation, transport, storage, exposure for sale and delivery of food of various kinds intended for human consumption; requiring wrappers or containers enclosing food of various kinds to be labelled or marked in accordance with the regulations; prohibiting or restricting the addition of any substance to, and regulating generally the composition of any food;

(20) control of milk and dairies;

(21) prescribing the special designations of milk;

(30) controlling the composition of bread, the addition of substances to flour, prescribing the descriptions under which, and conditions subject to which, flour may be sold; prevention of danger to health from the importation, preparation, transport, storage, exposure for sale and delivery of bread and flour;

(66) prescribing the qualifications of a public analyst.

The Minister of Agriculture and Fisheries may make regulations for:

(23) presuming evidence of adulteration of milk.

The regulations at present in force are the Sale of Milk Regulations, 1939; the Sale of Butter Regulations, 1902; the Dried Milk and the Condensed Milk Regulations, 1923 and 1927; the Public Health (Prevention of Tuberculosis) Regulations, 1925; the Milk and Dairies Order, 1926; the Imported Milk Regulations, 1926; the Milk (Special Designations) Regulations, 1936 to 1942; the Meat Regulations, 1924; the Preservatives in Food Regulations, 1925-27; the Shellfish Regulations, 1934; the Imported Food Regulations, 1937; the Public Analysts' Regulations, 1939; the Public Health (Preservatives in Food) Amendment Regulations, 1940.

Part I.—GENERAL PROVISIONS AS TO FOODS AND DRUGS.

(1) No person shall add, or direct or permit any other person to add, any substance to any food so as to render it injurious to health, or to any drug so as to affect injuriously the quality or potency, with intent that the food or drug may be sold in that state, and no person shall sell or possess any such food or drug.

(2) No person shall abstract, or direct or permit any other person to abstract, from any food any constituent thereof so as to affect injuriously the nature, substance or quality with intent that it may be sold in its altered state, unless notice is

given to the purchaser of the alteration, but in no case if the composition of the food thus treated fails to comply with any regulations made under the Act.

(3) If a person sells to the prejudice of the purchaser any food or drug not of the nature, or not of the substance, or not of the quality demanded by the purchaser, he shall be guilty of an offence, subject to the following section.

(4) The following defences are available to a person charged with an offence under the preceding section.

(i and ii) (a) That an addition or abstraction, in the case of a food, was not injurious to health, and in the case of a drug, has not affected injuriously the quality or potency; and (b) that the addition was not made fraudulently to increase weight, etc., or to conceal inferior quality; and (c) either that the addition was necessary for carriage or consumption, or that the article sold was labelled conspicuously with the name of the substance added or abstracted.

(iii) That the food or drug is the subject of a patent.

(iv) That the presence of some extraneous matter was an unavoidable consequence of the method of preparation.

(v) That the article supplied was a proprietary product supplied in response to a demand for that article.

(vi) That where the proceedings are in respect of diluted whisky, brandy, rum or gin that the spirit had been diluted with water only and that its strength was still not lower than 35 degrees under proof.

(6) (i) It is an offence for a vendor to give with any food or drug a label which falsely describes the article or is otherwise calculated to mislead as to its nature, substance or quality.

(ii) It is an offence to publish any advertisement which falsely describes or is calculated to mislead.

(9 to 12) Unsound Food. A person who sells, offers or exposes for sale, holds for the purpose of sale or of preparation for sale, deposits with or consigns to any person for the purpose or preparation of sale any food intended for but unfit for human consumption, is guilty of an offence. A person consigning unsound food can be proceeded against, and the consignee excluded from prosecution. Powers are given to inspectors to enter premises, examine food and if believed unsound seize and remove it in order to have it dealt with by a justice. The person from whom the food has been taken must be informed so that he may appear, when he will be entitled to be heard and to call witnesses. The justice may order the food to be destroyed or otherwise disposed of. If he refuses to condemn, the owner shall be compensated by the local authority for the value of any depreciation the food may have undergone. There is no right of appeal from the decision of a justice.

(13 to 16) Precautions against contamination of food. The occupier of premises is responsible for the observance of precautions against food contamination. In some cases, dealing chiefly with structural matters, the owner of the premises may be made responsible for carrying out the requisite precautions. Any person preventing the occupier or owner from complying with these requirements or permitting their contravention is guilty of an offence.

(14) Premises used in connection with the manufacture or sale of ice cream, the preparation or manufacture of sausages or potted, pressed, pickled or preserved food intended for sale including the preparation by cooking of meat or fish must be registered. Clubs, hotels and restaurants are exempt, and theatres, etc., are exempt unless ice cream is manufactured on the premises.

(17 and 18) Food poisoning. Medical practitioners are required to notify all cases or suspected cases of food poisoning to the Medical Officer of Health of the local authority. Suspected food may be detained by a Medical Officer until investigation is completed and if deemed unsafe is treated as unsound food (Section 10).

(19) No part of an animal slaughtered in a knacker's yard may be sold for human consumption.

Part II.—PROVISIONS AS TO MILK, DAIRIES AND ARTIFICIAL CREAM.

(24) No person shall add water, colouring matter, dried or condensed milk or liquid reconstituted therefrom to milk, or add separated milk or mixture of cream and separated milk to unseparated milk intended for sale for human consumption, or sell or have in his possession for sale any such milk. No person may sell as milk any liquid in which separated milk, dried or condensed milk forms a part.

(25) No person shall sell, or offer or expose for sale, for human consumption, or use in the manufacture of products for sale for human consumption, the

milk of any cow known to be tuberculous or suffering from certain other diseases.

(27) No person shall sell, etc., for human consumption under a designation including the word "cream," any substance purporting to be cream or artificial cream unless the substance is cream, or, if artificial cream, the word "cream" is immediately preceded by the word "artificial."

(28) Premises where artificial cream is manufactured or sold to be registered by Food and Drugs Authorities.

Part III.—PROVISIONS AS TO OTHER KINDS OF FOODS.

(32) No person shall sell, etc., any butter or margarine containing more than 16 per cent. of water, or any margarine, the fat of which contains more than 10 per cent. of fat derived from milk, or any milk-blended butter containing more than 24 per cent. of water. Any label or advertisement suggesting that margarine to which it relates contains butter, shall state the percentage of butter.

(33) Every person dealing in margarine, margarine-cheese and milk-blended butter must comply with certain requirements as to labelling.

(35) Occupiers of such factories, and wholesale dealers, must keep a register showing quantity and destination of each consignment and the register must be open to inspection by officers of the Ministry of Agriculture and Fisheries.

(36) If any substance intended for the adulteration of butter is found in a butter factory the occupier shall be guilty of an offence and any oil or fat capable of being so used shall be deemed to be intended to be so used unless the contrary is proved.

(37) Manufacturers of, and dealers in, ice-cream shall give notice to the medical officer of health of the occurrence of any milk-borne disease among persons living or working in or about the premises where the ice-cream is manufactured, stored or sold. If the M.O.H. suspects that any ice-cream is likely to cause any milk-borne disease, its sale or unauthorised removal may be prohibited and if on further investigation he is not satisfied that it may safely be used for human consumption, it may be destroyed.

(38) No person shall sell, etc., horseflesh for human consumption except in premises exhibiting a conspicuous notice and then only when asked for.

(39) Local authorities may provide apparatus for cleansing shell-fish.

Part IV.—PROVISIONS AS TO IMPORTATION.

(40) Importers of food which, in general, could not be sold without creating an offence under any Act or Regulations are guilty of an offence.

(41) Powers are given to the Commissioners of Customs and Excise to take samples and have them analysed by the Government Chemist. Samples are to be divided into three parts, one to be sent to the importer, one to the Government Chemist and one to be retained.

(42) The Commissioners of Customs and Excise are authorised to conduct prosecutions under this part of the Act, and the appropriate penalties are laid down. Should the analysis be disputed, the part of the sample retained may be sent for a joint analysis by the Government Chemist and some public analyst.

Part V.—MARKETS, SLAUGHTER-HOUSES AND COLD-AIR STORES.

(57 to 61) Slaughter-houses and knackers' yards. These premises are brought under control of the Local Authority. All licenses were to be cancelled by February 1st, 1940, and new licenses granted to suitable occupiers, in the first case for 13 months. Powers are granted to Local Authorities to provide public slaughter-houses, and if this is done private slaughter-houses may be eliminated and compensation paid. Local Authorities who provide markets or slaughter-houses are given powers to establish cold-air stores and refrigerators.

Part VI.—GENERAL AND MISCELLANEOUS.

(64) Defines "local authorities" and "foods and drugs authorities" and makes important changes in the local authorities who become food and drug authorities. The Council of a County District with a population of at least 40,000 at the last census becomes a Food and Drug Authority. On the application of a local authority with a population of at least 20,000 the Minister of Health may direct that authority to be the Food and Drugs Authority. On the application of a County Council that the formation of new Food and Drug Authorities in the county would render the duties of the Council difficult to carry out, the Minister may direct that the Council becomes the authority in place of the county district.

(66) The Food and Drugs authority must appoint one or more public analysts and may appoint a deputy analyst to act during any vacancy in the office of the public analyst or during the absence or incapacity of the public analyst. The appointment, terms of appointment and removal of the public analyst require the approval of the Minister of Health. No person may be appointed public analyst for any area who is engaged in any trade or business in that area connected with the sale of food and drugs.

(67) A county council or local authority may provide facilities for bacteriological and other examinations of samples of food and drugs.

(68) Defines a sampling officer and describes his powers. The sampling officer may take samples of milk at any dairy, at any time during transit, or at the place of delivery. Where milk sold in the area of one public authority is supplied from a dairy situated in an area of another public authority, provision is made to enable samples to be taken at the dairy on behalf of the original authority. (The Third Schedule to the Act requires the retailer to give the name and address of his supplier to the sampling officer if asked to do so. The retailer may demand that a sample of milk from the corresponding milking be taken for analysis provided that he gives the necessary notice to the local authority within sixty hours of taking sample; unless this is complied with proceedings cannot be taken against the retailer).

(69) A sampling officer may submit any sample of food or drug to the public analyst and the public analyst is required to make an analysis and submit a certificate in the prescribed form giving the result of his analysis. Any person may submit a sample of food or drug to the public analyst for the area in which the article was purchased; in this case the analyst may demand a fee not exceeding a guinea.

(70) A sampling officer who takes a sample with the intention of having it analysed must (1) inform the vendor that he intends to have an analysis made by the public analyst; (2) divide the sample into three parts and suitably mark and fasten up each sample; (3) give one part to the vendor, if required, send one part to the public analyst and retain the third part. Special provisions are laid down for taking a sample during the course of transit or from an automatic machine.

(72) The Minister of Health and the Minister of Agriculture are given powers to have samples of food purchased by their own sampling officers analysed by the public analyst. The samples are to be divided into four parts, of which one is retained by the Minister. The certificate is to be sent to the Minister who in his turn is to forward a copy to the local authority. The local authority must carry out any necessary proceedings and pay the analyst's fee.

(74) The public analyst must furnish quarterly reports to his authority stating the number of articles analysed and the result of each analysis, and the authority must transmit a copy of each report to the Minister.

(78) Penalties for obstruction. A vendor refusing to sell a reasonable quantity of an article exposed for sale is guilty of obstruction.

(80) No prosecution shall be commenced after the expiration of 28 days from taking the sample, but a magistrate may, on information given on oath, extend the time to 42 days. The "laying of information" is the first step in a prosecution. In any proceedings the summons shall not be made returnable less than 14 days from the day it is served; a copy of the certificate of analysis must be given with the summons.

(81) The public analyst's certificate is sufficient evidence of the facts stated on it: the analyst is not required to be called to prove his certificate, but he may be called for examination by either party.

(82) Gives power to have the third sample, which must be produced in court, analysed by the Government Chemist.

(83) States the defence available to a defendant when some other person is responsible for the commission of the offence charged.

(84) In any prosecution for selling, etc., an article not of the nature, substance or quality demanded it shall be a defence to prove (a) that the article was purchased as being of such a quality as would have so entitled him and with a written warranty to that effect; and (b) that at the time of the sale he had no reason to believe that it was otherwise; and (c) that it was sold in the same state as purchased. If this defence is to be adopted, the defendant must send a copy of the warranty to the prosecutor within 7 days of the summons being served, with a notice stating that he intends to rely on it and the name and address of the firm from whom the article was received. A name or description on an invoice shall be deemed to be a written warranty.

Alterations in the Composition of Food Due to War

Almost all articles used for food have been brought under control; a large number of Statutory Rules and Orders have been issued by the Ministry of Food under the Defence (General) Regulations dealing with the import, manufacture, grading, storing, distributing and sale of food. Definitions of various foods for the purpose of the Orders and descriptions of quality have been made and in several instances new standards laid down. The effect of these orders in some cases is to alter standards issued by the Ministry of Health, or to replace standards successfully enforced by custom or case law, though no part of the Food and Drugs Act is repealed. The Orders are enforced by Food Control Committees and suspected infringements are dealt with by the local Food Executive Officer, suspected samples being analysed by the Government Chemist. Food and Drug Authorities are necessarily concerned with the Food Orders in carrying out their statutory duties; proceedings must be brought under the Food and Drugs Act and the definition given in an Order is put forward as a presumptive standard.

The following are some of the Orders affecting the sale of articles of food:—

Bacon and Ham (40/633). Sodium or potassium nitrite may be added as a preservative, and articles of food containing bacon or ham as a constituent may contain sodium or potassium nitrite introduced with the preserved bacon or ham. (40/547.) Boron preservatives may be added to all cured pork except the offals, feet and head, and pickled pork.

Bread (43/42). Bread may not be prepared with dough containing more than 2 pounds of fat or oil to every 280 pounds of flour. Rye bread is bread the flour of which contains at least 25% of rye flour or rye meal (42/1223). Ingredients which may be used in the manufacture of bread include vinegar, acetic acid, acid calcium phosphate, sodium acid sulphate and sodium pyrophosphate (42/1363).

Bread, Biscuits, Buns, Cakes, Pastries, Rolls, Scones, etc. (41/431: 41/657: 41/1809). No milk, condensed milk, nor milk powder may be used in the manufacture of the above articles. A general licence (41/1809) authorises the use of skimmed milk powder in the manufacture of the above articles.

Cheese: Soft Cheese and Curd Cheese (41/431: 41/657: 41/945: 41/946). Definition: Soft cheese or curd cheese means cheese, the moisture content of which exceeds 55% (41/431), 50% (41/945). Condensed milk and milk powder may not be used in the manufacture of the above cheeses, but milk may be used by general licence.

Confectionery: Sugar Confectionery: Chocolate (41/431: 41/657). No milk, condensed milk or milk powder may be used in the manufacture of these articles.

Cream (40/1714). Definition: Cream produced from cows' milk, including sterilised cream (tinned cream). Manufacture and sale is prohibited except for making butter. Neither cream-cheese nor ice-cream made with cream may be manufactured or sold.

Cream, Synthetic (41/431). Definition: Any substance used as a substitute for cream including baker's filling. No milk, condensed milk or milk powder may be used in the manufacture of synthetic cream.

Dripping (41/606: 41/1889). Definition: Dripping means the clear unbleached and unadulterated fat, untreated by any chemical process, of sweet smell and produced by the rendering of fat and bones of sheep, oxen or pigs, the finished product to contain a minimum of 99% of saponifiable matter and a maximum of 2% of free fatty acids.

Flour Confectionery (42/2103). Cake shall not contain more than 20% of fat or 30% sugars, provided that either may be exceeded if the total does not exceed 45%.

Fruit Pulp (42/1650). Limitation imposed on the amount of water which may be added to fruit pulp. Limitation imposed on the amount of sulphur dioxide which may be added to fruit pulp; the amount in the product should not exceed 1500 parts per million permitted by the Preservative Regulations.

Horse flesh (41/1862). It is forbidden to manufacture or sell meat paste, soup, meat roll, galantine, ready or prepared meal, sausage, meat pie or other meat product or compounded article of food containing horseflesh.

Ice-cream (42/1692). The manufacture of ice-cream is prohibited.

Jam. See under marmalade.

Lemon Cheese and Lemon Curd (41/1639; 42/195). Lemon cheese and lemon curd may only be prepared under licence except in quantities of under 5 tons per annum. The percentage of soluble solids ascertained by a refractometer must not be less than 65%.

Margarine (40/982). A boron preservative may under licence be added to margarine or used in its manufacture. The amount added is usually about 0.20% calculated as boric acid (40/968). The order amends the conditions *re* labelling, in the Food and Drugs Act. Retailers may sell margarine in wrappers containing words other than margarine if in print not larger than the word margarine.

Marmalade (42/2482; 43/369). Definition: Any jam or preserve manufactured from citrus fruits with or without the addition of the juice or pectin of fruit other than citrus fruits. *Fresh Fruit Standard:* A specified fruit content varying with the variety; in the case of marmalade the fruit content is not less than 20%. To be made from fresh fruit or vegetables only. To contain no colour or preservative except sugar. *Full Fruit Standard:* The same fruit content as the above, but the requirement that the fruit or vegetables shall be fresh does not apply. *Special Standard Marmalade:* Jelly marmalade or coarse cut marmalade containing not less than 30% of fruit. In each case the jam or marmalade must contain not less than 68½% of soluble solids as determined by the refractometer.

Meat (41/1395). Meat may be preserved with sulphur dioxide by authority of the Minister.

Meat, Cooked Pickled (40/633). Cooked pickled meat other than bacon or ham may be preserved with sodium or potassium nitrite up to 200 parts per million calculated as sodium nitrite. Articles of food containing pickled meat as a component may contain nitrite introduced with the meat.

Meat Products (41/2021; 43/299). Prohibits the canning of meat or meat mixtures except tongues, certain varieties of soup, meat extract, meat or fish paste containing between 40 and 60% of meat or fish, meat rolls or galantine containing 30 to 45% of meat, or any ready or prepared meal containing the same; also certain mixtures of pork and beans. The five varieties of soup permitted to be canned must conform to a minimum standard of quality for solids protein and/or fat.

Milk (41/431; 41/657). The use of liquid milk, condensed milk or milk powder is forbidden wholly or in part in the manufacture of the following specified articles: Biscuits, Bread, Buns, Cakes, Pastries, Rolls, Scones, etc.; Ice-cream; Soft Cheese and Curd Cheese; Sweetmeats; Synthetic Cream.

Rice (42/1276). Manufacture except under licence of flaked rice and rice cones; retail sale of rice flour, ground rice, granulated rice and rice cones prohibited. Rice may not be used in the manufacture of any bread or meat product, seasoning, or "black" or "white" puddings.

Saccharin (42/2455). Mixtures containing flour or starch with saccharin or dulcin, sweetening powders, saccharin or dulcin solutions, may only be prepared under licence. It is illegal to buy or sell by retail saccharin, soluble saccharin, saccharin solution, dulcin solution or sweetening powder. Saccharin solution for pharmaceutical use is permitted under general licence (42/1773). Saccharin may be sold in packets of 100 tablets, each weighing not more than 1.1 gr. and containing between 0.18 and 0.22 gr. of saccharin and not more than 5% of water-insoluble matter (42/1106).

Sardines, Canned (41/671). Grade I. In pure olive oil with or without tomatoes. Grade II. In oil other than pure olive oil with or without tomatoes.

Sausages (41/62; 41/220; 41/709). Definition: Beef sausages include sausages the meat in which is not beef alone. Pork sausages are to contain at least 90% of pork in the meat. Beef sausage meat and pork sausage meat are defined in similar terms. Sausages other than pork (or Kosher beef) are to be deemed to be beef sausages and similarly for sausage meat. Standard: Sausages and sausage meat must have a meat content between 30 and 45%.

Soft Drinks (42/1337). Controls the manufacture of liquids drunk without dilution and of concentrated articles, liquid, semi-solid or solid drunk after suitable dilution.

Soya Flour (42/863). Soya flour may only be manufactured under licence. Mixtures containing soya flour, other than food substitutes manufactured under licence, may only be manufactured under licence. A general licence (42/180) permits the use of soya in sausages and other products.

Starch Food Powders (41/1742). Definitions: Cornflour: Starch in powder form suitable for use in the preparation of human food. Starch means starch made from maize, potato, rice, rye, wheat, sago, tapioca, arrowroot. Blancmange Powder and Custard Powder: Starch with or without other farinaceous matter blended with flavouring, colouring or other ingredients. The manufacture of Cornflour, Blancmange Powder or Custard Powder may only be carried on under licence.

Manufactured and Pre-Packed Foods (Control) Order S.R. & O. 42/1863

This Order called for a comprehensive return to be rendered to the Ministry of Food in respect of the manufacture and pre-packing of a wide variety of foods, prohibited the manufacture and pre-packing of any food in respect of which no return was made (i.e., of any new food and of certain foods exempted from the requirement), and made provision for making these operations subject to control by licence so far as such categories of foods as might be specified from time to time are concerned. The returns called for by the Order provided the Ministry with details of the composition of all manufactured foods as defined which were then on the market. Under this Order licences are at present (March 1943) required for the manufacture and pre-packing of suet and shredded suet made from premier jus (42/2073); any food containing gelatin, isinglass or agar and one or more of the following: sugar, glucose, saccharin or other sweetening agent, starch, tartaric acid, citric acid, flavouring, gum, arrowroot, flour or any other cereal (42/2476); any food containing 25% or more of flour (with certain exceptions) (43/68).

NOTES ON WATER ANALYSIS

Sampling

A sample of water for analysis should be taken in a thoroughly clean Winchester which has been rinsed out two or three times with the water to be analysed. If tap water is concerned allow the water to run for several minutes and then fill a bottle completely; if the sample is to be taken from a river, lake, well, cistern, etc., immerse the Winchester completely and then withdraw the stopper. The container should be marked with the date and source and the water sent for analysis as soon as possible after sampling.

Physical Examination

Odour: No odour should be perceptible even on warming.

Turbidity: Note whether it is bright and clear or opalescent or turbid. If turbidity is pronounced, filter 1 litre through a weighed Gooch crucible, dry at 100°, weigh and express the result in parts per 100,000.

Colour: Note in a 100 ml. Nessler tube, comparing with distilled water. A yellow tint may indicate organic contamination.

Conductivity: The greater the saline content the higher the electrical conductivity. The E.C. of a very soft water may be ten times that of distilled water, while that of a hard water may be 300 times as great. The conductivity at 20°, expressed in gemmhos (1.0×10^{-6} reciprocal ohm) divided by 20 roughly approximates to the degrees of hardness.

Chemical Examination

Total Solids. Heat a clean platinum basin of about 70 ml. capacity to 180° for fifteen minutes, cool in a desiccator and weigh. Transfer to the basin, in successive amounts, a quantity of the sample varying from 100 ml. to 500 ml. and evaporate to dryness on a steam bath. On completion, wipe the outside of the basin and dry to constant weight at 180°. The amount of sample used should be such that the residue weighs not less than 0.02 g. Drying at 180° is adopted to avoid discordant results due to retention of water of crystallisation; even at this temperature calcium sulphate retains 1 molecule. In reporting results, the temperature of drying should always be stated. Ignition of the residue may yield an indication of the presence of organic matter and the ignited residue may be analysed qualitatively to detect the presence of Na, K, Ca, Mg, Fe, Mn, Pb, Zn or other metals.

Total solids should not exceed 50 parts per 100,000.

Ammonia. *Free or saline ammonia* (usually in combination with carbonic acid).

1 litre distillation flask with the delivery tube bent down at right angles and fitted by means of a cork into an upright, double surface condenser which itself dips into a 50 ml. measuring cylinder used as a receiver.

Place 500 ml. of the sample, together with 1 g. of freshly ignited Na_2CO_3 , in a flask and distil fairly slowly and regularly over a free flame, collecting three 50 ml. portions in separate measuring cylinders. Nesslerise the *second* fraction first with standard ammonium chloride solution (1 ml. \equiv 0.01 mg. NH_3). If less than 2 ml. of the standard is required, the whole of the first portion may then be treated similarly; if more than 2 ml. is used, half of the first portion should be diluted to 50 ml. and then Nesslerised. The total volume of ammonium chloride used for the whole distillate is equivalent to the "free" ammonia in 500 ml. of sample; calculate as parts per 100,000.

Albuminoid Ammonia. This determination serves only as a rough guide to the quantity of organic matter in solution, as only a variable amount of the total organic nitrogen is recovered as ammonia.

Dissolve 4 g. of potassium permanganate and 100 g. of sodium hydroxide in about 700 ml. of water and boil until the volume is 500 ml. Add 50 ml. of this solution to the water remaining in the flask after the determination of free ammonia, add some pieces of ignited porous pot to prevent bumping, and continue the distillation, collecting a further four 50 ml. fractions. Nesslerise the whole of this distillate, beginning with the last portion, and calculate the amount of albuminoid ammonia per 100,000.

More than 0.005 part per 100,000 of free ammonia indicates possible pollution; more than 0.01 part per 100,000 of albuminoid ammonia is rarely encountered in satisfactory water.

Nessler's Reagent for Ammonia. *Syn.* SOLUTION OF POTASSIO-MERCURIC IODIDE.

Dissolve potassium iodide 7 and mercuric chloride $2\frac{1}{2}$, in distilled water 160. To this add more of the mercuric chloride in solution until the precipitate no longer disappears on well stirring, and a slight permanent precipitate remains. Then add sodium hydroxide 24, dissolve, add a little more solution of mercuric chloride and distilled water, *q.s.* to 200.

Nessler's Reagent (Richmond's Formula).

A very sensitive reagent may be made by mixing a solution of 17.5 g. of potassium iodide in 100 ml. of water with 15 g. mercuric chloride in 300 ml. of water, thoroughly washing precipitate by decantation, and dissolving in 17.5 g. of potassium iodide in 100 ml. of water. A few drops of mercuric chloride solution are then added until a precipitate insoluble on shaking is formed; the mixture is diluted to about 500 ml., cooled in ice and mixed with a solution of 105 g. sodium hydroxide in 250 ml. The mixture is made up to 1 litre and allowed to settle.—H. D. Richmond, *per Pharm. J.*, ii/1925, 394.

The following formula is recommended.—45.5 g. of mercuric iodide and 34.9 g. of potassium iodide are dissolved in a minimum of water, 112 g. of potassium hydroxide added (140 ml. of an almost saturated solution, *sp. gr.* 1.538), and the volume made up to 1 litre. It should be allowed to stand for several days to allow any precipitate to settle. The solution is 0.2N with respect to the mercury content. In the Nessler test, 5 ml. to 100 ml. of final volume is used and the colour comparisons made with the standards 30 minutes after mixing.—A. P. Vanselow, *Industr. Engng Chem. (anal. Edn.)*, 1940, 516.

Estimation of Ammonia in Water in presence of Hydrogen Sulphide. The presence of hydrogen sulphide in a water interferes with the Nessler test. If the amount of ammonia be large the sulphide may be precipitated with a zinc or lead salt and the ammonia can then be estimated directly by the Nessler reagent. If the amount is small it is best to add to 500 ml. of the water a measured quantity of N/1 sulphuric acid and distil 100 ml.—this completely removes H_2S . A volume of N/1 NaOH equal to that of the H_2SO_4 used is now added. The water is again distilled until 200 ml. have been collected and the Nessler test is applied to the distillate.

Chloride. The average content is about 2 parts per 100,000, though frequently one finds a content of 5 to 15 parts per 100,000. It should be remembered that urine and sewage are, comparatively speaking, highly charged with chlorine—this enables the analyst to determine whether a high albuminoid ammonia content is attributable to sewage or vegetable influence. *Per contra*, almost

entire absence of chlorides, coupled with excess of albuminoid ammonia and little free ammonia, suggests vegetable contamination of a dangerous character. One frequently obtains waters for examination with an exceedingly high Cl-content in conjunction with an almost total absence of organic impurity. Such waters, though "saline," are suitable for drinking purposes.

Place in a white porcelain basin 100 ml. of the sample and 1 ml. of a 5% solution of potassium chromate. Titrate with standard solution of silver nitrate (1 ml. \equiv 1 mg. Cl), with frequent shaking, to a faint, permanent reddish-brown colour. Then titrate with the sample until this colour is *just* destroyed (up to 20 ml. may be required). The amount of silver nitrate used is equivalent to the chloride in 100 ml. plus half the extra water used in the second titration. If the sample is acid, add a little sodium bicarbonate; if it contains small amounts of hydrogen sulphide, add a crystal of zinc sulphate before titration. The reagents must be Cl-free.

Oxygen Absorbed. Pure waters absorb very little oxygen whereas those that are polluted may absorb comparatively large quantities, and hence this determination, whilst difficult to interpret in terms of type or quantity of organic matter, has considerable value.

Place 250 ml. of the sample in a clean stoppered bottle, warm to 37°, add 10 ml. of N/80 potassium permanganate, 10 ml. of 25% H_2SO_4 , and keep at 37° for three hours. Cool quickly, add potassium iodide and titrate the excess of permanganate with N/250 sodium thiosulphate recently standardised. From the amount of permanganate decomposed by the water calculate parts of oxygen absorbed per 100,000 (1 ml. N/80 solution of potassium permanganate \equiv 0.1 mg. of available oxygen).

Different laboratories use vastly different times and temperatures in this determination: comparisons are valid only if based upon figures obtained under identical conditions. The figures obtained should be considered in relation with those for albuminoid ammonia and should be repeated if the ratio of oxygen absorbed to albuminoid ammonia differs materially from 10 to 1.

Nitrites. Rarely present in natural waters except in minute traces, and a qualitative test is all that is required.

ILOSVAY'S REAGENT. Dissolve 0.1 g. of α -naphthylamine in 120 ml. of boiling distilled water, cool and add 30 ml. of glacial acetic acid. Dissolve 0.5 g. of sulphanilic acid in 120 ml. of distilled water and 30 ml. of glacial acetic acid. Mix the two solutions and keep in a stoppered bottle. If any colour develops on keeping, add a little zinc dust, and filter.

Test. Add 2 ml. of the above reagent to 50 ml. of the sample in a Nessler cylinder. The appearance of a pink colour in a few seconds denotes more than a trace; if it takes 10 minutes to develop, only a minute trace is present. Alternatively, the amount present may be determined by conducting the test under Nesslerising conditions, using a standard solution of sodium nitrite as a standard.

Alternative Method. The reagent consists of dimethylaniline 1 g.; sulphanilic acid 1.5 g. in N/2 hydrochloric acid 100 ml. 2 drops added to 10 ml. of a solution containing nitrites gives a red coloration. In the presence of very small quantities of nitrite 1 ml. of reagent is used, the solution being allowed to stand for 10 minutes. Under these conditions a nitrite can be detected in a 1 in 1,000,000 solution. The colour is due to methyl orange.—J. C. Giblin and G. Chapman, *Analyst*, 1936, 686.

Nitrates. A determination of nitrates should always be made. First apply a qualitative test by carefully mixing 1 ml. of the sample with 3 ml. of nitrogen-free sulphuric acid; cool and add brucine; a bright red is obtained if 10 parts per 100,000 are present—a pale rose if only 0.1 part.

Place from 10 to 100 ml. of the sample, depending upon the amount of nitrate present (10 ml. if 1 part per 100,000) in a small flask with a few grammes of thoroughly washed zinc-copper couple, acidify with a few drops of 10% hydrochloric acid and heat at 37° for one hour. Place about 300 ml. of distilled water in the distillation apparatus used for the determination of ammonia together with 1 g. of ignited sodium carbonate, and distil 50 ml. to remove all ammonia. When the reduction is complete, pour the water from the copper-zinc couple, together with the rinsings of the couple with distilled water, into the flask and distil two fractions of 50 ml. Determine the amount of ammonia in the fractions by Nesslerising and calculate the number of parts of nitrate (as NO_3) in 100,000.

Alternative Method. Determination of nitrates in water is best carried out with phenoldisulphonic acid after removal of chlorides, if present in quantities above 50 mg. per litre, by adding silver sulphate in the quantity theoretically necessary, tartrate being added to prevent precipitation of salts of Ca and Mg. After filtering, the liquid is evaporated to dryness and 2 ml. of phenoldisulphonic acid added, and the mixture made up to 100 ml. with 2 ml. of Fehling's solution No. 2, 8 ml. of 50% potassium hydroxide. The colour produced is compared with standards. The phenoldisulphonic acid is prepared by heating 25 g. of phenol with 150 ml. of concentrated sulphuric acid and 75 ml. of fuming sulphuric acid.—E. Remy and H. Enzenauer, *Arch. Pharm., Berl.*, 1936, 274, 435.

Determination of Nitrates in Presence of Chlorides. 10 ml. of sample is evaporated to dryness with 1 ml. of 1% sodium salicylate solution. To the residue, cooled in a desiccator, add rapidly 1 ml. of sulphuric acid and, after mixing, 10 ml. of water and 10 ml. of ammonium hydroxide solution. The colour produced is compared with that obtained with a standard nitrate solution similarly treated.—H. Caron and D. Raquet, *J. Pharm. Chim., Paris*, 1936, 23, 446.

Hardness. The “degrees” of hardness refer to the soap-destroying power, 1° being roughly equal to that of 1 part of calcium carbonate, or its equivalent in other calcium or magnesium salts, in 100,000. “Temporary” hardness is that which disappears on boiling and is due to bicarbonates; “permanent” hardness is that which remains after boiling. No diminution in hardness occurs on boiling unless more than 2° is due to bicarbonates, as calcium carbonate is soluble to that extent. As the hardness is due to bicarbonates, carbonates, sulphates and chlorides of calcium and magnesium, the correspondence between degrees and the content of these salts is only approximate.

Water-softening. Water may be softened by the addition of only 2 parts per million of sodium hexametaphosphate, after filtration. It is completely non-toxic.

Total Hardness. Place 100 ml. of the sample in a 200 ml. stoppered bottle and titrate with standardised soap solution until, after shaking vigorously, the lather persists for five minutes, the bottle being laid on its side. Subtract 1 ml. from the reading, as

this amount is required to produce a lather in water free from salts: the net number of millilitres of soap solution used is equal to the degrees of hardness. If more than 15 ml. of soap solution is required, repeat the titration, using 50 ml. of sample diluted to 100 ml. with distilled water: the degrees of hardness will then be twice that of the dilution.

Temporary Hardness. Boil 100 ml. of the sample in a flask of resistance glass for 30 minutes, replacing that lost by evaporation from time to time with distilled water. Allow to cool, filter, make up to 100 ml. and determine the degrees of Permanent Hardness by titration with soap. Total Hardness minus Permanent Hardness equals Temporary Hardness.

Standard Soap Solution. Dissolve 10 g. of hard soap in 1 litre of 45% alcohol. To standardise this solution, dissolve 1 g. of calx spar in slight excess of hydrochloric acid, evaporate to dryness and dissolve the residue in sufficient distilled water to make 1 litre. Dilute 10 ml. of this solution to 100 ml. with distilled water and titrate with the soap solution. Adjust the soap solution until 11 ml. is the amount required.

Degree of hardness (total)—

| | | | | |
|----------|-----|-----|-----|-------------|
| 5° | ... | ... | ... | Soft waters |
| 15° | ... | ... | ... | Hard waters |
| Over 20° | ... | ... | ... | Very hard |

If the hardness is over 30° the water is unfit for general purposes.

Poisonous Metals. Concentrate the water 5 times after acidifying with a few drops of hydrochloric acid. Add ammonia and hydrogen sulphide solutions. A darkening in colour shows the presence of Pb, Cu, or Fe, but not Zn. Divide into two portions; acidify one part with hydrochloric acid. The disappearance of the colour indicates the absence of Pb and Cu. To the second part add potassium cyanide solution—the persistence of the colour shows that it is due to the presence of Pb. Confirmatory tests should always be employed.

Iron. Acidify 50 ml. of sample with sulphuric acid and add potassium permanganate solution until a slight pink colour persists. Filter and add solution of potassium ferrocyanide solution. If iron is present, a blue colour is formed.

Zinc. If present, an opalescence will be noticed in the above test. A pure soft water may exert a solvent action upon zinc (e.g., galvanised kettles), and may become dangerous to health.

Copper. If present, a reddish-brown colour is obtained with the ferrocyanide test.

Lead. Acidify 100 ml. of sample with acetic acid and concentrate to 5 ml. Filter and add a crystal of potassium chromate. In presence of lead, a precipitate of lead chromate is obtained.

EXCESSIVELY PURE WATER may be solvent of lead in service water. It is recommended to harden it by adding lime. PEATY WATERS owing to acidity often dissolve lead from main pipes in the form of lead hydrogen carbonate. On standing or on boiling, it is thrown out with the calcium carbonate.

Determination of Lead in Potable Waters. A volume of sample containing 0.05 to 0.1 mg. of lead is evaporated to small volume in a Pyrex flask and 1 ml. of sulphuric acid and 1 ml. of perchloric acid are added. Heating is continued until organic matter is destroyed and the excess of perchloric acid is driven off. The liquid is cooled and the following added in order:—10 ml. of water, 1 ml. of glacial acetic acid, 5 ml. of 20% solution of sodium citrate free from lead, and 5 ml. of strong solution of ammonia. The volume is then adjusted with water to 25 ml. A blank is prepared in exactly the same way without addition of the sample, the sulphuric and perchloric acids being heated until the latter is driven off. 5 to 10 ml. of the liquid obtained from the sample

is measured into a 50 ml. volumetric flask and similar amounts of the liquid from the blank are transferred to each of another two volumetric flasks, to one of which is added 1 to 2 ml. of standard lead acetate solution (0.1831 g. of lead acetate, $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 3\text{H}_2\text{O}$, in 5 ml. of glacial acetic acid and water to 1 litre, and diluted 1 in 10 when required for use so that 1 ml. = 0.01 mg. of Pb). To each flask is now added 6 drops of 5% sulphurous acid, 5 ml. of 1% solution of potassium cyanide PbT, 10 ml. of carbon tetrachloride and 0.5 ml. of 0.1% solution of diphenylthiocarbazon in carbon tetrachloride. The contents of each flask are poured into test-tubes, the aqueous layers are pipetted off and the unchanged diphenylthiocarbazon is removed by repeated shakings with 5 ml. portions of the potassium cyanide solution. The pink carbon tetrachloride solutions of the lead complex are then washed once with water and the colours compared in a colorimeter. The best depth of colour for colorimetric comparison is that corresponding to 0.1 to 0.3 mg. of lead. Bismuth and stannous tin also form complexes with the reagent but they are extracted when the carbon tetrachloride solutions are shaken with the cyanide solution. Other metals do not interfere. If iron is present in quantity, the lead must be separated and for this purpose sodium diethyldithiocarbamate is suitable. After treatment with sulphuric acid and perchloric acid followed by addition of acetic acid, sodium citrate and ammonia as above, the mixture is transferred to a separating funnel, the volume of mixture and washings amounting to 30 to 40 ml.; the pH must be above 9. The liquid is then shaken with 2 ml. of 2% aqueous sodium diethyldithiocarbamate and 25 ml. of ether, the separated ethereal extract is washed with 10 ml. of water and is then transferred to a 300 ml. Pyrex flask, the separating funnel being rinsed with 5 ml. of ether. The aqueous solution is extracted with two further quantities of ether in the same way. The ether is then evaporated off, the organic matter destroyed by treatment with sulphuric and perchloric acids and the determination continued as described above.—S. L. Tompsett, *Analyst*, 1936, 591.

Calcium and Magnesium. Acidify 200 ml. of sample with 1 ml. of dilute hydrochloric acid and concentrate to 50 ml.; make alkaline with ammonia, add 2 ml. of saturated solution of ammonium oxalate and heat for one hour. Collect and thoroughly wash the precipitate, dissolve in dilute sulphuric acid and titrate with N/100 potassium permanganate (1 ml. = 0.0002 g. Ca). Magnesium is determined in the filtrate and washings from the above estimation after making the volume up to 100 ml. Of this, take a volume containing approximately 0.5 mg. of magnesium (say 25 ml.) and dilute to 100 ml. in a Nessler glass; add 2 ml. of ammoniacal solution of ammonium phosphate and agitate for 2 minutes with a plunger. Compare the opalescence with that produced by 100 ml. of a solution of magnesium sulphate of known concentration treated similarly.

Fluorine. Fluorine may be determined colorimetrically using a reagent made by mixing 3 ml. of a solution of zirconyl chloride (3.53% w/v of $\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$) with 1 ml. of 1% aqueous sodium alizarin monosulphonate and diluting with water to 200 ml. To 50 ml. of the sample in a Nessler glass add 2.5 ml. of hydrochloric acid, mix, and add a sufficient quantity of the reagent to give a very faint pink tinge remaining after standing for 10 minutes. For fluorine contents below 1 part per million, 2 ml. of reagent is sufficient; for 5 parts per million, 4 ml. of reagent is required, the tint being orange. The colour produced is matched against standards obtained by treating similarly and at the same time solutions containing 1, 2, 4 and 8 parts per million of fluorine (as fluoride).—Guy Barr and A. L. Thorogood, *Analyst*, 1934, 378.

Removal of Fluorine. The use of bone filters for the removal of fluorine from water is now practicable for both large and small water supplies. Bone may be prepared for use by boiling with alkali and neutralising with acid; a better method is calcination of the bone at 400° to 600° for 10 minutes, followed by treatment with acid for 10 minutes. The bone is regenerated by alkali and acid. A particle size of 40–60 mesh has been found desirable. The capacity of the bone to remove fluorine is directly proportional to the period of contact; slow rates of flow are therefore desirable. It appears that the removal of fluorine is due neither to a true chemical reaction nor to adsorption, but occurs by the formation of solid solutions after partial displacement by fluorine of either the hydroxyl or carbonate group of the treated bone. It has been found that 1 cu. ft. of bone (40–60 mesh) will treat 4900 gal. of water containing 3.5 p.p.m. fluorine before regeneration. In South Africa this is done on a large scale by adding 2 gal. of a highly concentrated aqueous solution of

superphosphate to 1000 gal. of water and gradually adding powdered slaked lime to precipitate the phosphate. Excess lime can be precipitated by forcing carbon dioxide through the water.—per *Bull. Hyg.*, 1941, 402.

Fluorine and Mottled Teeth. A daily intake of 0.1 to 0.15 mg. of fluorine per kilogramme of body-weight is sufficient to cause mottling. Sufficient fluorine may be removed from water to render it harmless by coagulation with aluminium sulphate and filtration.—*Lancet*, ii/1934, 34.

About 90% of the children born and bred in Maldon, Essex, were found to have mottled teeth due to the presence of between 4.5 and 5.5 parts per million of fluorine in the water supply. The essential condition is that the water should be drunk during the years the teeth are being formed. When once through the gums, the condition could not be influenced one way or the other.—N. J. Ainsworth, *Analyst*, 1934, 380.

Observations made at Ennis, Texas, show that prior to 1926 no mottling of children's teeth was noted. In this year the city water supply was changed to a deep well which had a fluorine content of 4.4 parts per million. A careful survey shows that 98% of the children born and living in Ennis since then have this disfigurement caused by mottling of the enamel. Mottled enamel can be prevented in 100% of cases by using only distilled or rain water, or water that is known not to contain any fluorine, for drinking and cooking purposes from birth to at least seven years of age, to include those cases where the development of teeth is abnormally slow.—C. A. McMurray, per *Bull. Hyg.*, 1935, 759.

Studies on the minimal threshold of the dental sign of chronic endemic fluorosis (mottled enamel).—H. T. Dean and E. Elvove, *Publ. Hlth Rep., Wash.*, 1935, 1719.

A number of samples from different sources of the Board's supply were examined during 1935 but in only one instance (Campsbourne Well) was the amount present in excess of minimum concentration associated with "mottled teeth," namely, 0.7 to 1 part per million.—*Rep. metrop. Wat. Bd.*, 1935.

Amounts of fluoride not exceeding 1 part per million are of no public health significance.—H. T. Dean, *Publ. Hlth Rep., Wash.*, 1937, 1263.

Mottled teeth have been found in nearly 22% of 5019 men and women examined. In most cases the mottling was slight. Of those living in the London area 23% were affected. The highest proportion was in those from Hertfordshire and Northampton (85%).—L. Spira, *Lancet*, i/1942, 649.

Iodine. The quantity of iodine in natural waters is infinitesimal although deep wells may contain more than surface water. No definite conclusions have been reached concerning the correlation between the absence of iodine in water and the prevalence of endemic goitre. It has been observed that the iodine content of water in Michigan is inversely proportional to goitre incidence and a similar relationship has been shown to exist in New Zealand between iodine content of soil and goitre incidence. On the other hand, water of high calcium content may interfere with iodine absorption and thus contribute a positive factor in the incidence of goitre (see Leader, *Brit. med. J.*, i/1931, 361). The earth's crust and not the sea is held to be the storehouse of iodine and heavy cropping of the soil tends to deplete it of iodine (*Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 123, 1929).

According to the Netherlands Goitre Commission it is necessary, if goitre is to be prevented, that 80 to 100 mg. of iodine are ingested daily. On this basis the food produced in Holland is not up to standard, especially outside the larger towns, where the people live on the products of their own locality. The content of the drinking water in iodine is most important and is the determining factor in this matter of sufficiency of iodine intake.—P. A. Meerburg, per *Bull. Hyg.*, November 1935, 716.

The question has received much attention and the reader is referred to Vol. I, page 49, for further information on this subject, and for details to the following literature:—

Iodine in Nutrition: A Review of Existing Information, J. B. Orr and I. Leitch, *Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 123, 1929 and No. 154, 1931; O. P. Kinball, *J. Amer. med. Ass.*, ii/1931, 1877; *Endemic Goitre in Switzerland*, A review of recent contributions to its aetiology, incidence and prevention. Olesen, *Publ. Hlth Rep., Wash.*, 1933, 651.

Interpretation of Results.

Before a final judgment can be delivered upon any water there have to be taken into consideration (1) its geological history,

(2) the rainfall before and after collection, (3) the method of storage and distribution, (4) the surface drainage, and (5) a bacterial examination. A water which chemically is organically pure may be bacterially contaminated, and on the other hand a bacterially pure water may be chemically dangerous or suspicious.

Bacteriological Examination of Water

It should be emphasised that the bacteriological examination of water should only be attempted by those who have had training and experience in this branch of work.

The enumeration of the bacteria present is not so important as the determination of the species. Many bacteria are natural to water and quite harmless, but those which have their origin in sewage or manure are always dangerous. Search should be made for various sewage-pollution organisms, e.g., *B. coli communis*, *B. typhosus*—especially the *B. coli* group—*Vibrio cholerae*, *B. proteus*, *B. enteritidis sporogenes* Klein, and streptococci.

Results should be considered in relation to the history and geological source of the sample. The waters from deep sources have been subjected to a natural process of filtration and are often purer than surface waters but in some formations, such as chalk, fissures develop which render the filtration insufficient.

Enumeration of Bacteria. Prepare agar and gelatin plates with varying quantities of the specimen, e.g., 1·0, 0·1 and 0·001 ml., and incubate the former at 37° for 24 hours and the latter at 22° for 72 hours. The more plates prepared the greater the accuracy, but three of each is a reasonable number. The amount of sample used depends upon the purity of the sample; a very pure water might require the use of 3 ml. The colonies may be counted by drawing sector lines with a paraffin pencil through the petri dish, counting one section, and multiplying out to obtain the number of bacteria in the entire amount of water taken for examination. *Pakes' Discs* are employed in a similar manner. To obtain accurate results it is important to add the melted gelatin or agar medium to the specimen of water, and not the water to the medium. This procedure ensures better mixing.

The plates should be examined at intervals, and if liquefying organisms are numerous (which suggests sewage pollution) the examination has often to be concluded in a shorter time than would be necessary where such are not present.

Cultivation on gelatin at 22° enumerates the bacteria present normally in the water, whilst the body temperature (37°) will be more suitable for excremental organisms derived from, or pathogenic to, the animal body.

B. coli communis. The following may be regarded as decisive tests for *B. coli*. (1) Acid and curd in litmus milk; (2 and 3), motility and indol in peptone water; (4) negative to Gram's stain; (5) no liquefaction with streak cultures on gelatin; (6) fluorescence on rebiel agar; (7, 8 and 9) fermentation of sucrose, mannite and dulcitol respectively.

Pollution with sewage is usually indicated by the presence of the *B. coli* group of organisms. Acid and gas production in **MacConkey's Litmus Bile Salt Glucose Broth Medium** gives presumptive evidence of the presence of *B. coli*, *B. paratyphosus*, *B. enteritidis*—but excluding *B. typhosus* and the dysentery organisms. These latter produce acid formation only (without gas) in this medium.

Another step is to employ the MacConkey medium made with **lactose** instead of glucose—this forms a useful corroboration for *B. coli*—this organism gives acid and gas whereas none of the others do so. In tabular form the matter may be stated as follows:—

| | Glucose | Lactose | Motility | Gelatin | Litmus Milk. 3 days | Indol. |
|-------------------------------------|---------|---------|----------|---------|------------------------|--------|
| <i>B. coli communis</i> ... | A.G. | A.G. | + | — | A.C. | + |
| <i>B. typhosus</i> ... | A. | — | + | — | A. | — |
| <i>B. paratyphosus</i> ... | A.G. | — | + | — | Alk.* | — |
| <i>B. enteritidis</i> (Gaertner)... | A.G. | — | + | — | Alk. | — |
| <i>B. dysent.</i> (Shiga) ... | A. | — | — | — | Alk. | — |
| <i>B. dysent.</i> (Flexner) ... | A. | — | — | — | Alk. | + |
| <i>B. Morgan</i> No. 1 ... | A.G. | — | — | — | Alk. | + |

A. = Acid. G. = Gas. C. = Clot. — under gelatin means non-liquefaction.

* *Vide* also Eyre, Bact. Technique, for *B. paratyphosus* A and B.

The method used aims at the determination of the smallest quantity of water which will give a positive reaction followed by the application of a number of confirmatory tests for *B. coli*. Varying quantities of water are used for each test, in order to obtain a series which contains both positive and negative results. Different dilutions of the following special broth are prepared so that preparations made with varying quantities of the sample will all have approximately the same concentration: 1 of broth and 2 of a mixture of sample and freshly distilled water.

MacConkey's Glucose Broth

| | | | | |
|----------------------|-----|-----|-----|---------|
| Sodium taurocholate | ... | ... | ... | 1.59 g. |
| Dextrose | ... | ... | ... | 1.59 g. |
| Peptone | ... | ... | ... | 6.09 g. |
| Water, distilled, to | ... | ... | ... | 100 ml. |

Boil for 20 minutes, adjust to pH 7.5 with N/1 NaOH, reboil and add sufficient litmus to produce a decided colour on dilution with two parts of water. This broth is also prepared using lactose instead of dextrose.

The exact mode of procedure for the detection of acid and gas formation is as follows:—

Examination of 50 ml. of the water. Place 50 ml. of a mixture of 2 parts broth and 1 part distilled water in a 100 ml. flask containing a small inverted test-tube rendered bubble-free. The whole is then plugged with sterile wool and sterilised at 100° for 20 minutes on 3 successive days. This has to be made ready before receipt of the specimen. 50 ml. of the sample to be examined is introduced with aseptic precautions. The same procedure is applied with the *lactose* preparation.

Examination of 20 ml. of the water. Take 10 ml. of broth in a 6×1 in. test-tube containing a small inverted test-tube rendered bubble-free and after sterilisation add 20 ml. of sample.

Examination of 10 ml. of the water. Take 10 ml. of a mixture of 2 parts of broth and 1 part of distilled water and treat similarly.

Examination of 5 ml. of the water. Take 10 ml. of equal amounts of broth and distilled water.

Examination of 2 ml. and less. Take 1 part of broth and 2 parts of distilled water.

All the tubes prepared are incubated at 37° for 48 hours, and the production of turbidity, acidity and gas indicates the presence of *B. coli*.

Subsequent to these results inoculate Neutral Red Bile Salt Agar plates with loopfuls from the cultures in the bottles—using a “spreader” made with a piece of glass rod $\frac{1}{8}$ inch diameter with an end bent at right angles to the handle and about 1½ inches long.

After incubating for 24 hours pick out with a platinum loop colonies resembling those of *B. coli*, and inoculate sloped agar tubes, *thence* peptone water for the Rosindol reaction and Indol reaction—also litmus milk for the “Acid and Curd,” and examine a fresh broth culture for motility.

The plate cultures are incubated further to observe fluorescence, if any.

Rosindol Reaction (Ehrlich's). *Syn.* Böhme's Indol Test. To 10 ml. of a 48-hour peptone water culture add 5 ml. of the following solution:—

| | | | |
|--------------------------------|-----|-----|----|
| p-Dimethylaminobenzaldehyde... | ... | ... | 1 |
| Alcohol, 96% | ... | ... | 95 |
| Concentrated hydrochloric acid | ... | ... | 20 |

and then 5 ml. of saturated aqueous potassium persulphate solution. Shake well. According to MacConkey 1 ml. of each solution is sufficient. Pink colour in a few minutes = +. In some cases the persulphate need not be added. The pink colour is soluble in amyl alcohol—a little of which should be added, especially in doubtful cases. At least 48 hours' growth should be allowed; in some cases 6 to 8 days are required.

Indol Reaction. To 5 ml. of the (6 or 7 days) peptone water culture add 1 ml. of concentrated sulphuric acid and then 1 ml. of 0.02% sodium nitrite. Pink colour indicates indol production (some organisms, e.g., cholera vibrio, do not require the sodium nitrite—hence the test may be done in two stages). It may be necessary to incubate for 8 days or more before conducting the test.

The data in question, together with the production of fluorescence in the colonies in Reibel Agar, *syn.* MacConkey's Neutral Red Bile Salt Agar—which has the composition:—

| | | | | | |
|-----------------------------|-----|-----|-----|-----|-----------|
| Agar and peptone white | ... | ... | ... | ... | aa. 30 g. |
| Lactose | ... | ... | ... | ... | 15 g. |
| Sodium taurocholate | ... | ... | ... | ... | 7.5 g. |
| Tap water | ... | ... | ... | ... | 1500 ml. |
| Solution of neutral red, 1% | ... | ... | ... | ... | 7.5 ml. |

constitute the “Flaginac” reaction which is typical of *B. coli*.

This word is made up to show the reactions on these media and is applied to organisms, e.g., *B. coli*, which will respond to all:—

- fl: fluorescence in neutral red.
- ag: acid and gas formation.
- in: indol in peptone water.
- ac: acid and clot in litmus milk.

Neutral Red, $C_{11}H_{12}N_4$ (*syn.* Toluyene Red), is chemically dimethyldiaminoethylphenazine hydrochloride. It is readily soluble in alcohol and in ether.

The presence of sulphur bacteria, *Beggiatoa alba*, which are readily identified, may be used for detecting sewage pollution, in place of a *B. coli* count.—Prof. Davis Ellis, *Pharm. J.*, ii/1926, 308.

***B. typhosus*.** Thresh states that “the search for pathogenic bacteria, such as typhoid-paratyphoid-salmonella bacilli, in water is beset with difficulties, and is rarely successful.” “Water supplies which naturally, or after treatment, are practically free from *B. coli* can safely be considered innocent of spreading

either typhoid, salmonella or dysentery infection." The isolation of *B. typhosus* involves a concentration by flocculation with aluminium sulphate, followed by centrifuging or by filtration through a suitable filter. The next stage consists in the culture of the bacteria in a medium favourable to the growth of the typhoid-paratyphoid bacilli and inhibitory to others. Final identification rests upon the determination of the morphological and biochemical properties of the cultures obtained. Sir A. Houston (*Rep. metrop. Wat. Bd.*, 1930) states that Wilson's researches have simplified the search for these organisms.

Wilson's Sulphite-Bismuth Medium: Dissolve 6 g. of bismuth ammonio-citrate scales in 50 ml. of boiling distilled water and neutralise with about 2 ml. of 10% solution of sodium hydroxide. Mix with a solution obtained by boiling 20 g. of anhydrous Na_2SO_3 in 100 ml. of water to which, whilst boiling, 10 g. of anhydrous Na_2HPO_4 have been added. When cool, add 10 g. of glucose dissolved in 50 ml. of boiling distilled water and add 20 ml. of this preparation to 100 ml. of a hot melted 3% nutrient agar, then 1 ml. of 8% aqueous ferrous sulphate solution and finally 0.5 ml. of 1% solution of brilliant green in distilled water. Pour into petri dishes and inoculate the surface when set. *B. typhosus* and *B. paratyphosus* appear as flat, black colonies, the former within 24 hours, the latter within 48 hours.—Wilson, *Brit. med. J.*, ii/1933, 561.

BACTERIOLOGICAL REPORTS ON WATERS

If *B. coli* forms a considerable proportion of the total number of organisms present there is great reason to suspect sewage pollution of human or other animal origin. Generally speaking, a water containing *B. coli* in 50 ml. but not in less is quite good if the count of total bacteria and the chemical analysis are good.

Wells, Shallow and Surface. If chemical results and surroundings are bad, even if *B. coli* be absent from a large volume of the water, it should be condemned, and *per contra* if in a suspicious locality the bacteriological examination is bad the water ought to be condemned even though chemically it could be passed.

The usually accepted standards for a surface water are:—

- B. coli* absent in 10 ml.
- Streptococci* absent in 10 ml.
- B. Welchii* absent in 100 ml.
- Total colonies at 37° = 50 per ml.
- " " " 22° = 500 "

Wells, Ordinary or Medium Depth. *Total Bacteria.* The gelatin count may show from 100 to 2000 organisms per ml. The presence of *B. coli* in 10 ml. would condemn the water.

Wells, Deep. *Total Bacteria.* Should not exceed 100 bacteria per ml. Artesian wells and some springs may contain very small amounts, e.g., 5 or 10 organisms per 100 ml.

Presence of *B. coli* in 100 ml. or less cannot be permitted.

Rivers. Draw conclusions as under Wells (Shallow). Content varies enormously with season. *Total Bacteria.* The gelatin count varies enormously. *B. coli* in 10 ml. would condemn.

CHEMICAL AND BACTERIOLOGICAL EXAMINATIONS OF DRINKING WATERS COMPARED

A water may pass certain chemical standards and yet be unsatisfactory from the bacteriological aspect. The converse may also be true in some cases. The table on the following pages, prepared from results obtained by Martindale, illustrates this point. The waters comprise a selection as supplied from the main to consumers in some of the leading cities and health resorts in Great Britain, including, e.g., London, Glasgow, Bath, Blackpool, Buxton, etc.

CHEMICAL AND BACTERIOLOGICAL EXAMINATIONS OF DRINKING WATERS

| Source | Chemical | | | | Bacteriological | | | | | | | | | | Conclusions |
|----------------|----------------------------|-------|----------------------------|---|------------------|--------------|---|------------------|-------------------|----------------|------------------------------------|------------------------------|---|----------|--|
| | Ammonia, parts per million | | Chlorine parts per 100,000 | Solids parts per 100,000 and effect on ignition | Bacteria per ml. | | Vol. producing Acid and Gas in MacConkey with | | Rosindol Reaction | Indol Reaction | Fluorescence on Reibel-agar plates | Acid and Clot in Litmus Milk | | Motility | |
| | | | | | Gelatin at 20° | Agar at 37°* | Glu-cose† | Lac-tose† | | | | Acid Clot | | | |
| | | | | | | | | | | | | | | | |
| Water No. 1 | Nil. | 0.06 | 1 | 6 Much charred | 100 | 160 | ml. 10 | ml. 10 | + | + | + | + | + | + | Chem., good. Bact., not satisfactory. |
| No. 2, Bath .. | 0.02 | 0.034 | 2 | 36 slight charring | 972 | 8 | 100 | 100 50 acid only | - | - | - | + | + | + | Chem., excellent. Bact., satisfactory. |
| No. 3 .. | Nil. | 0.06 | 1.5 | 12 slight charring | 1209 | 57 | 10 | 50 | - | + | + | + | + | + | Chem., good. Bact., satisfactory. |
| No. 4, Buxton | 0.03 | 0.12 | 1 | 15 much charring | 45 | 80 | None with 100ml. | None with 100ml. | - | - | - | - | - | - | Chem., safe. Bact., excellent. |
| No. 5 .. | 0.026 | 0.056 | 5 | 45 v. sl. charring | 172 | 199 | 10 | 10 | + | + | + | + | + | + | Chem., good. Bact., not satisfactory. |

| | | | | | | | | | | | | | | |
|---------------------------|-------|-------|-------|----------------------|---|---------------------------------------|------|-----|----|---|---|---|---|--|
| No. 6 ... | Nil | 0.026 | 3 | 30 not charred | 1109 | 37 | 50 | 50 | — | — | + | + | — | Chem., excellent. Bact., satisfactory |
| No. 7 ... | ... | 0.026 | 0.03 | 1 | 3 charred | at least 1000 some liquefng. | 820 | 10 | 10 | — | — | — | + | Chem., excellent. Bact., might be better. |
| No. 8 ... | ... | 0.08 | 0.12 | 1.5 | 18 charred | at least 1000 some liquefng. | 1600 | 50 | 50 | — | — | — | — | Chem., safe or- ganically. Bact., might be better. |
| No. 9 ... | ... | 0.01 | 0.036 | 6.5 | 45 v. sh. charring | 1040 | 242 | 50 | 50 | — | — | — | — | Chem., excellent. Bact., satisfac- tory. |
| No. 10, Village Well A | 0.08 | 0.168 | 10.5 | 70 charred | 10 | 60 | 1 | 10 | + | + | + | + | + | Chem., unsatis- factory. Bact., bad. |
| Ditto B | 0.026 | 0.12 | 10.5 | 68 charred | B. sub- stis pre- vented count | 75 | 100 | 100 | + | + | + | + | + | Chem., unsatis- factory. Bact., satisfac- tory. |
| No. 11 | Nil | 0.11 | 1.5 | 6 much charred | 3000 | 50 | 10 | 10 | + | + | + | + | + | Chem., safe or- ganically. Bact., unsatis- factory. |
| No. 12* London A ... | Nil | 0.04 | 1 | 32 | less than 10 | 20 | 100 | 100 | + | — | + | + | + | Chem., excel- lent. |
| London B ... | Nil | 0.03 | 1 | 32 | Nil. | 21 | 100 | 50 | + | + | + | + | + | Bact., satisfac- tory. |

* West London Main 2.29 Cl per 100,000 and Solids 37 per 100,000.—W. H. Martindale, January 16th, 1929.

| Source | Chemical | | | Solids parts per 100,000 | Chlorine parts per 100,000 | Ignition and effect on per 100,000 | Bacteriological | | | | | | Motility | Conclusions |
|--|----------------------------|-------|------------------|--------------------------|----------------------------|------------------------------------|---|-------------------|----------------|------------------------------------|------------------------------|---------------|----------|---|
| | Ammonia, parts per million | | Bacteria per ml. | | | | Vol. producing Acid and Gas in MacConkey with | Rosindol Reaction | Indol Reaction | Fluorescence on Reibel-agar plates | Acid and Clot in Litmus Milk | | | |
| | Free | Alb. | Gelatin at 20° | | | | | | | | | Agar at 37° * | | |
| | | | | | | | | | | | | | | |
| No. 13 | Nil. | 0.08 | 2 | 21 slight charring | 1400 | 1350 | ml. 10 | ml. 10 | + | + | + | + | + | Chem., good. Bact., not satisfactory. |
| No. 14, Margate | Nil. | 0.04 | 2.5 | 30 not charred | 800 | 30 | 10 | 10 | - | - | - | - | - | Chem., excellent. Bact., satisfactory. |
| No. 15, Norfolk (Private well before repair) | 0.026 | 0.076 | 4 | 40 | 52 | 32,000 | 1/100 | 1/100 | + | + | + | + | + | Chem., org. safe. Bact., bad. |
| Ditto (after repair) | 0.1 | 0.07 | 4 | 36 | 70 | 157 | 10 | 50 | - | - | - | + | + | Chem., org. safe. Bact., improved, now safe, subject supervision. |

* Pathogenic and intestinal organisms grow best at this temperature.

† Presumptive evidence of *B. coli*, *B. paratyphosus*, *B. enteritidis*, but excluding *B. typhosus* and dysentery organisms.

‡ Confirmatory for *B. coli* since *B. typhosus*, *B. paratyphosus*, *B. enteritidis* and dysentery organisms do not give it.

Columns 7 to 13 include the "Flaginac" reaction.

Columns 9, 10, and 14 show results of cultures in peptone water from least quantity of MacConkey's Culture showing acid and gas.

The results suggest that (1) Neither bacteriological nor chemical examination is adequate individually but together they constitute an adequate safeguard for the purity of water; (2) the absence of *B. coli* from 100 ml. of a water is an ideal seldom attained; (3) the albuminoid ammonia content is no indication of the number of bacteria, but in conjunction with chlorine, nitrite and nitrate data may suggest sewage contamination; (4) examination of waters at the source and after traversing some miles of water supply pipes may show marked differences.

CHLORINATION OF WATER

Chlorination is carried out either after or before filtration. In some cases a large excess of chlorine is added to destroy algæ as well as bacteria, the excess being removed by means of SO_2 . Chlorine produced by electrolysis of sea-water is used in some parts instead of chlorine cylinders. In France, eau de Javelle is employed. Probably the most effective method is the use of ammonia and chlorine in the proportions of 1 to 4 or 1 to 8, in a small volume of water which is then added to the water in bulk. The increased efficiency is due to the formation of a chloramine compound. The general trend of opinion appears to favour chlorination after filtration. Removal of any objectionable taste may be effected by the addition of potassium permanganate in doses of 0.2 to 0.8 parts per million, before, with, or after the chlorine treatment. Results obtained on New River Water (*Rep. metrop. Wat. Bd.*, 1931) illustrate results obtained by this treatment: of 238 samples, 62% showed *B. coli* in 1 ml. or less before chlorination and no *B. coli* in 71% of the samples after chlorination.

The enteric fever mortalities per 100,000 of Paris, London and Berlin for 1929 were 4.1, 1.0, and 0.9 respectively. Thanks to Philippe Bunau-Varilla and his advocacy of the chlorine process of water sterilisation, to which he has given the term "verdunisation," Paris in 1932 has at last achieved a reasonably safe supply of drinking water.—*Lancet*, ii/1932, 590.

It is shown that an increase of pH from 2.8 to 8.2 reduces the power of water (containing some organic matter in the form of hay infusion or meat bouillon) to combine with chlorine. Preammonisation enhances this reduction considerably. The prevention of the chlorophenol taste by means of preammonisation depends upon the pH and can only be achieved at a pH of 7.0 or more. At a lower pH, ammonia has no effect. The bactericidal power of chlorine in a preammonised water is greater than with chlorine alone but is reduced with increase of pH, although the effect of a lower hydrogen ion concentration is less marked if periods of contact of one hour or more are permitted. From the practical standpoint it is, therefore, important to maintain the water that is being purified at a pH of about 7.0 in order to secure both an adequate bactericidal effect and the prevention of chlorophenol taste.—M. L. Koschin, per *Bull. Hyg.*, 1935, 802.

A plant was put down at Ottawa in which chloramine was used. Although slightly more expensive, it is stated to have the advantage of preventing after-growth, which is a serious problem on the Continent. A tablet containing 6 mg. will disinfect about 25 fl. oz. of water in a few minutes, the only taste imparted to the water being that of chlorine.—C. R. Downs, *Industr. Engng Chem.*, 1934, 26, 20.

Owing to a great development of algal growths in the New River during the summer of 1934 the experiment was tried of using "cuprichloramine," a compound of chlorine and cuprammonium ($\text{Cu}(\text{NH}_4)_2$). For general purposes a combination round about 0.3 copper sulphate, 0.25 Cl_2 , and 0.15 NH_3 appeared to give good results, but in view of a possible loss of ammonia, as it passed down the New River before meeting the chlorine, it was decided that a trinity would

best serve the purpose, namely, $0.3 + 0.3 + 0.3$. On August 6th samples from the New River showed the presence of *Cyclotella*, *Pandorina* and *Synedra*. Within 24 hours after the adoption of the cuprichloramine treatment the water had changed from an opaque grassy colour at Bush Hill (where the cuprammonium was added) to a slaty blue at Hornsey (where the chlorine was added). The effect of the treatment was also obvious on the water when it passed into the Green Lane Reservoir where it showed up as a light grey-blue, and from August 14th centrifuged samples from the reservoir proved entirely free from algal growth. The most striking result, however, was that the bacteriological returns for the Hornsey works reached and maintained a standard which has never been attained before. The general conclusion reached, as a result of these experiments, was that the presence of ammonia, copper and chlorine in water in appropriate proportions was conducive to the best germicidal and algicidal results.—C. H. H. Harold, *Rep. metrop. Wat. Bd.*, 1934.

Rapid Method for the Determination of Free Chlorine in Chlorinated Water. 20 ml. are placed in each of two or three test-tubes and acidified with hydrochloric acid. Three or four crystals of potassium iodide and 3 to 5 drops of 1% starch solution are added. The formation of a blue colour indicates the presence of free chlorine, the concentration of which can be determined from colorimetric standards. Transverse and longitudinal examinations of ten tubes (on a white background) show respectively the following colours with the given concentrations of free chlorine in mg. per litre: 0.05, no colour and light sky-blue; 0.1, light sky-blue and sky-blue; 0.2, light sky-blue and light blue; 0.4, sky-blue and blue; 1.0, light blue and dark blue.—V. A. Peshka, per *Bull. Hyg.*, 1941, 16, 584.

Further Methods of Sterilisation

The Excess Lime Method of treating water is effective for sterilising and purifying, and when necessary softening water.

The bactericidal dose is about 1 to 2 parts of CaO per 100,000 parts of water, the excess lime being removed by means of carbon dioxide. If the consumers of London water generally demanded a softened water, were prepared to pay for it, and Parliamentary sanction could be obtained, investigations have proved that the method is sound on chemical, bacteriological and physical grounds.—Sir A. C. Houston, *Rep. metrop. Wat. Bd.*, 1930.

The Southend Waterworks Company initiated the Excess Lime Method, in 1929 at Langford, to provide a supply of 7,000,000 gallons of purified and softened water per day, the raw water being both extremely hard and also contaminated. The results are excellent, no *B. coli* being found in 100 ml. of the treated water, the hardness averaging 10 parts per 100,000, albuminoid ammonia being reduced from 0.0245 to 0.0041 parts per 100,000 and the oxygen absorbed in 3 hours at 37° reduced from 0.235 to 0.060. A detailed description of the plant and method of operation.—*Municipal Review*, March, 1930, per *Rep. metrop. Wat. Bd.*, 1930.

Silver has been used, the action being due to solution of the metal (1 in 20,000) (Kling, *Brit. med. J. Epit.*, ii/1932, 14). The silver probably forms an association with the bactericidal protein (Leader, *Lancet*, i/1934, 93). Water may be rendered sterile and bactericidal by filtration through porcelain candles previously heated at 1200° after having silver chloride fused into their medium. The bactericidal effect is lost after 5 days.—Lakhovski, *C.R. Acad. Sci., Paris*, 1932, 194, 137.

The Catadyn Process is based upon the action of minute amounts of metals. The vessels consist of filtering devices with an active silver surface. 21 million germs per ml. of water killed in 48 hours. For special purposes and household use the method may have a considerable future but is too expensive for employment on a large scale.—Biggar and Griffiths, *Brit. med. J.*, ii/1932, 883; *Pharm. J.*, ii/1929, 87; *Rep. metrop. Wat. Bd.*, 1930.

Disadvantages of the contact process, such as the size of container necessary for treating large volumes of water or the formation of incrustations on the silvered surfaces, are avoided in the Electro-Catadyn process in which silver ions liberated by passing a weak electric current between silver electrodes are caused to enter the water. By regulating the strength of the current the optimum amount of silver ions for any particular purpose can be admitted to the water. The water is passed through an activator containing a number of silver electrodes.—A. Karsten, per *Bull. Hyg.*, Nov. 1935, 722.

Ozone has been utilised abroad, but is far more expensive than chlorine.

A large number of experiments were carried out on the ozone treatment of water, a plant, capable of dealing with 5120 gallons per hour, having been installed at Barn Elms (*Rep. metrop. Wat. Bd.*, 1931). Bacteriologically the results were perfect and the treatment improved the water chemically and physically. The treatment is more expensive than chlorination (0.260d. per 1000 gallons).

Undoubtedly ozone imparts an excellent finish to water and is a definite remover of the majority of tastes. On the other hand ozone itself imparts a specific penetrating odour and taste to water if not desaturated by cascading and is said by some to be more nauseating than chlorine. From the point of view of the consumer ozone presents the greater attraction and if properly applied is an effective sterilant provided that at all times an absence of breaches in the filter barrier can be guaranteed. On the other hand chloramine offers an all-round security under every possible condition and is the "safety-first" of waterworks practice. On all ozone-operated works chlorination is held in reserve as an alternative measure. Since future commitments demand the maintenance of large impounded reserves at risk with greater possibility of algal troubles appearing, a combination of coagulation and ozonisation presents most attractive features worthy of special consideration.—C. H. H. Harold, *Rep. metrop. Wat. Bd.*, 1934.

Horrocks's Water Testing Method is used to determine the amount of bleaching powder required to sterilise the contents of an army water-cart.

The method uses zinc iodide, or potassium iodide and starch solution, as reagent.—Compare *Field Sanitation* by Moor and Cooper (Baillière, Tindall and Cox).

The test automatically adjusts the strength of the purifier to be used to the particular water to be treated. The Horrocks's Test Case contains 6 white enamelled tumblers (170 ml.) and 1 black one (250 ml.). Bleaching powder—a levelled scoopful of about 2 g.—is rubbed fine and dissolved in the black tumbler filled to the inside mark. The white tumblers are filled with the water to be tested. One drop (1/15 ml.) of bleaching powder solution is added to No. 1, two drops to No. 2, up to 6 drops in No. 6. These are stirred and left for 20 minutes, when about 6 drops of a stock solution of potassium iodide and starch are added to each. A blue colour will indicate that after all organic matter has been destroyed an excess of available chlorine remains whereby iodine is liberated, with the formation of the blue iodide of starch. The number of the first mug of the series which shows a definite colour, gives the number of scoops of the bleaching powder required to sterilise the contents of one water-cart (110 gallons approx.). The powder should be dissolved before adding it to the water-cart, and contact for one hour should be allowed, before the water is issued to the troops.

Alum Box, in the army water-filtering cart, contains a mixture of alum 75% and dry sodium carbonate 25%. By the action of the water, aluminium hydroxide is formed and deposited on the filter cloth, the jelly-like mass formed imitating the natural zooglyca layer of a sand filter-bed.

Emergency Drinking Water. Seriously contaminated water can be purified by the addition of a small quantity of free chlorine, added in the form of bleaching powder or as sodium hypochlorite. Probably the most convenient preparation for wide distribution is *Liquor Sodæ Chlorinata*, B.P.C., diluted 1 in 2.5 so as to produce a solution containing about 1 per cent. of available chlorine. Such a solution can be made by dissolving 3 oz. of sodium carbonate in 5 fl. oz. of water, triturating 2 oz. of chlorinated lime with 15 fl. oz. of water, mixing the two liquids, allowing to stand (with an occasional shake) for three or four hours, filtering and diluting to 50 fl. oz. Directions: Add 10 drops to each pint of water; stir; allow to stand five minutes; add a small crystal of hypo, and when this has dissolved the water can be used. "Hypo" is a commonly employed "anti-chlor.", that is, it will remove free chlorine from a solution, forming sodium chloride and a little free acid.

When chlorinated soda solution is diluted with water to produce a 1% solution of chlorine, the dilution should be done before filtration, because of the heavy precipitate of calcium carbonate formed.—R. J. Stratton, *Pharm. J.*, i/1941, 250.

SOME BRITISH SPAS

In the following list of the more important spas the approximate proportion of the principal constituents in parts per million of the

water, and the season for each spa are indicated. However, in summer and autumn all are in season. Spring is a favourable season for waters, especially in children, while chilly, elderly and rheumatic people are often most benefited by autumn and winter treatment. A course of baths will often fully restore the functions of the skin, while both baths and waters increase the natural elimination of waste products, and appropriate baths can increase the efficiency of the heart muscle and stimulate or soothe the circulatory and nervous systems. Besides these effects in regulating disordered functions and a certain intensive influence in chronic disease, spa treatment is also alterant, and is therefore beneficial in digestive, rheumatic and arthritic diseases.

The treatment at different spas is suited to two constitutional types of chronic illness:—Type A. Deficiency of body heat; chilly subjects with poor circulation, languid and anæmic, circulatory hypotension. Type B. Excess of body heat; warm subjects, florid and congested, hyperpiesia, circulatory hypertension.

Bath (SOMERSET).—One of the warmest winter resorts. Climate sedative, reinforcing the effect of thermal treatment; cooler and more invigorating on the Downs, 200 feet above the city. Hyperthermal radioactive gaseous waters; diuretic, solvent, promoting elimination of uric acid; pools and manipulation douches, aeration and vapour baths. *Indications:* Type B, most rheumatic conditions with inactivity of the skin and circulation requiring *thermal* heat and manipulation and re-education; indolent and indurated skin affections, constipation and chronic forms of colitis, certain functional and degenerative conditions of the heart, hepatic dysfunctions—inhalation of natural radioactive gas for catarrhal and gouty affections; season all the year. Calcium sulphate 1400, sodium sulphate 300, magnesium chloride 200, calcium carbonate 100, strontium, iron, silica, aluminium and bromine, gas 52 parts by weight, mainly nitrogen and carbon dioxide with argon, neon, helium and niton, 104° to 120°.

Buxton (DERBYSHIRE).—Climate very tonic, bracing, dry and keen, pleasantly cool in summer, atmospheric humidity low owing to dry subsoil. Gaseous sub-thermal nitrogenous waters, diuretic, alterant; also chalybeate. Pools at 83° and at 98°, aeration, manipulation and peat baths. *Indications:* Type A, rheumatic conditions, arthritis and periarthritis, especially in anæmic and depressed rheumatic subjects; fibrositis, gout, the uric acid diathesis, urinary gravel and calculus and some cardiac cases, convalescence, anæmia, asthma and malaria. *Contra-indications:* Unsuitable for respiratory catarrhs, heart disease or nephritis, debilitated subjects with poor reactive qualities. Season, May to September. Calcium carbonate 200, sodium chloride 40, iron and manganese; gas—nitrogen 59·8%, carbon dioxide 40·2%.

Cheltenham (GLOUCESTERSHIRE).—One of the most sheltered inland climates of the British Isles; sedative. Sulphated salt waters with some alkali, mildly laxative, metabolic. Manipulation douche, aeration, brine and paraffin wax baths. *Indications:* Type B. Gastro-intestinal and cardiovascular disorders, acid dyspepsia, constipation, hæmorrhoids, glycosuria and obesity, gastro-hepatic and bronchial catarrhs, high blood pressure and degenerative changes of the blood vessels, convalescence, insomnia. Season, all the year round. Four springs. Fieldholme or "Twin-salt"—sodium sulphate and magnesium sulphate each 3800, calcium 1500. Lansdowne—sodium chloride 5600, with half as much sodium sulphate. Pittville or "Hepatic water"—sodium bicarbonate 400. Chadnor or "Renal water"—magnesium and calcium saline.

Droitwich (WORCESTERSHIRE).—Fairly equable climate, with remarkable freedom from fog and snow; tonic-sedative, the general atmosphere restful although not relaxing; summers pleasantly warm, winters not cold. Saturated brine radioactive baths for stimulant surface treatment, sub-thermal and thermal pools, aeration and effervescent baths and douches. *Indications:* Type A. Fibrositis, sciatica, lumbago, neuritis, rheumatoid arthritis and osteoarthritis, paralysis, functional weakness of heart and circulation with low blood pressure,

peripheral stasis. Season, all the year. Sodium 120,690, magnesium 132, calcium 1368, chlorine 185,550, bicarbonate 26, sulphate 4491, radon 0.2 m.m.c. per litre and 12.4 m.m.c. in the dissolved gases.

Harrogate (YORKSHIRE).—Very tonic, bracing climate with freshness and elasticity of the air, relative humidity low, winters cold but not bleak, summers tempered and fresh. Strong and milder sulphur-saline waters, laxative, stimulant, metabolic and saline, chloride of iron and pure chalybeates, deposits of medicinal mud. Sulphur, saline, douche, vapour and peat baths. *Indications:* Type B. A tonic spa for hyperpiesis, hepatic disorders, glycosuria, constipation, arterial hypertension, congestive arthritis of middle life, menstrual disorders and chronic malaria. Season, spring, summer and autumn. Saline—sodium chloride 13,300, sodium sulphide 150, barium chloride 100, hydrogen sulphide 37,000 volumes. Alkaline sulphur—sodium carbonate 450, sulphide and very little salt. Tonic chalybeate—saline and iron carbonate 150, and stronger with iron carbonate 500.

Leamington (WARWICKSHIRE).—Mild, equable and dry climate; sedative. Strong hypertonic salt-sulphate water with predominance of chlorides and calcium, aperient and diuretic, and mild saline, hypotonic water, diuretic. Saline, aeration and Turkish baths. *Indications:* Type B. Digestive and toxic disorders, chronic autotoxæmia, obesity, arterial hypertension. Season, spring to autumn. Strong saline—sodium chloride 12,451, calcium sulphate 2842, magnesium sulphate 377.

Llandrindod Wells (RADNORSHIRE).—Pleasant and invigorating, tonic-sedative air. Mild saline springs—hypotonic, saline sulphur water—solvent, eliminant, metabolic and chalybeate waters. Saline, douche and aeration baths. *Indications:* Type A. Chronic gastro-intestinal catarrhs and toxæmias, mucous colitis, rheumatic disorders and asthenic states. Season, summer and autumn. Saline—sodium chloride 1800 to 6200, calcium chloride 400 to 1300, lithium chloride and carbonate 1000. Salines with hydrogen sulphide 7000 to 9500 volumes.

Moffat (DUMFRIES-SHIRE).—Very pure and equable air, bracing especially during the spring and autumn—tonic-sedative. Hypotonic, saline sulphur waters, diuretic, alterant, also an uncommon form of iron water in Hartfell mountain, 4 miles away, for debility and anæmia. Sulphur baths. *Indications:* Type A. Chronic rheumatism and gout, atonic indigestion and tropical liver, chronic toxæmias and bladder and renal cases where diuresis is indicated, convalescence. Season, summer and autumn. Sodium 392, calcium 61, magnesium 25, barium 15, strontium 9, potassium 4, lithium 0.5, chloride 721, bicarbonate 141, sulphate 14, bromine 7, sulphide 7, nitrate 3, iodide 0.2 and silicic acid 19.

Strathpeffer Spa (ROSS-SHIRE).—The most northerly of the British spas. Sheltered, tonic-sedative climate, but fresh and invigorating on the upper slopes; essentially a tonic spa with its cool, long summer days. Strong non-aperient sulphur waters with calcium, diuretic, alterant, also a chalybeate for neurasthenia and anæmia. Sulphur, douche and peat baths, inhalations. *Indications:* Type A. Atonic, digestive or nervous conditions, chronic senile rheumatism, arthritis, obscure toxæmias, bone and skin affections of toxic origin, hepatic congestion and mucous colitis. *Contra-indications:* Acute rheumatic and gouty disorders, persistent high blood pressure. Season, summer and autumn. Calcium sulphate 271 to 729, calcium carbonate 100 to 214, magnesium sulphate 570 in one water, hydrogen sulphide 18,000 to 69,000 volumes per million.

Woodhall (LINCOLNSHIRE).—One of the driest climates in England, fresh and bracing air. Hypertonic bromo-iodine salt waters, unsuitable for internal use without dilution, metabolic, eliminant. Salt baths, fango packs and inhalations for respiratory diseases. *Indications:* Type A. Osteoarthritis, muscular rheumatism, fibrositis and peripheral neuritis, diseases peculiar to women, chronic glandular enlargements, exophthalmic goitre, Graves' disease. Season, summer and autumn. Sodium chloride 14,700, calcium chloride 3600, sodium sulphate 1300, bromine 50 and iodine 24.

For particulars of other Spas and Marine and Inland Health Resorts see "British Health Resorts," edited by R. Fortescue Fox (and also for details of New Zealand, South African and Canadian resorts).

BACTERIOLOGICAL AND CLINICAL NOTES

with Reference to Special Diseases.

Abortus Fever. Abortus fever is the variety of undulant fever occurring in England. It is primarily a disease of animals, communicable to man, characterised by fever of long duration and undulant character and is caused by *Brucella abortus*. The *Brucella* group of organisms comprises:—*Br. abortus* (bovine); *Br. abortus* (porcine) (*Br. suis*); *Br. melitensis*. All may cause undulant fever in man. In Great Britain, undulant fever in man is always due to infection with the bovine organism (see Undulant Fever, p. 986).

In a suspected case of abortus fever, a leucocyte count should be made; this usually shows a leucopenia with relative lymphocytosis. Cases showing leucocytosis are probably not abortus fever and some other cause for the pyrexia should be sought. The serum of patients usually gives a positive agglutination reaction to *Br. abortus* by the tenth day of the disease. A titre of 1:100 or over is considered diagnostic; in all cases the test must be carried to high dilutions because there is often an inhibition zone, which may extend to a dilution as high as 1:640, in which no reaction occurs. Positive blood cultures are obtained in only about 16% of cases and incubation must be conducted in an atmosphere containing 10% of CO₂ (see also p. 988).

Cultivation from Spinal Fluid. 1 ml. of a 1:100 dilution of inactivated polyvalent antibrucella rabbit serum was mixed with 5 ml. of the spinal fluid and incubated for 30 minutes. The spinal fluid serum mixture was then centrifuged at high speed for 15 minutes and the sediment planted on liver infusion blood agar slants and grown at 37° under increased carbon dioxide tension. To accomplish the latter, the upper portion of the test-tube was heated in the flame to force out some of the air, the expired breath of the bacteriologist was blown into the tube through a sterile plugged pipette and a rubber stopper immediately inserted.

Growth appeared in tubes inoculated from the mixture of spinal fluid and serum within 24 hours, whereas cultures of the untreated spinal fluids, some of which were centrifuged, showed no growth on the same medium until after 72 hours.—M. A. Poston and D. T. Smith, *New Engl. J. Med.*, ii/1936, 370.

Prophylaxis of *Br. Abortus* Infection in Cattle. Prophylactic inoculation with a dead vaccine has proved useless. The correct use of a live vaccine made from a known attenuated strain of *Br. abortus* does not produce a lasting infection of cattle nor lead to the excretion of infective bacteria in the milk, while it gives substantial protection against even a virulent strain of the organism. The Ministry of Health (S.R. & O. 1942, No. 771) has now sanctioned the use of a live vaccine prepared and used according to methods approved by the Ministry of Agriculture. Vaccine prepared from "strain 19" is to be preferred for calves as it is effective in a single dose; "strain 45," of which two doses are required, is reserved for adult animals as it has the advantage of being "rough" and not producing agglutinins to confuse subsequent tests for immunity.—*Lancet*, i/1942, 621.

Acne Vulgaris. The acne bacilli (*Corynebacterium acnes*) are gram-positive organisms which, when seen in pus, are arranged very irregularly. The bacilli are found in approximately 50% of cases of acne vulgaris. They stain less deeply than the cocci and grow with difficulty on artificial media.

Cultivation. A suitable medium for growing the organism is nutrient agar containing from 1 to 5% of oleic acid. Good results may be obtained by growing anaerobically in broth for 3 weeks and then plating on serum agar with neutral red and about 2% of oleic acid.

It can also be grown in deep tubes of 2% glucose agar, the reaction of the medium being distinctly acid. Whitish colonies appear after 3 or 4 days at 37° which under a low magnification show a lenticulate shape. The relation of the bacillus to the suppuration in acne has been a matter of dispute.

Sudmerson and Thompson use an acid serum agar taking the deeper parts of the comedo in which the bacillus usually predominates, emulsifying this in saline and spreading thinly on the slope so as to obtain colonies to pick off.

The acne bacillus is less sensitive than the staphylococcus to penicillin and by using a concentration of penicillin which inhibits staphylococci a pure culture of acne bacilli can be obtained direct from an acne lesion. A tube of glucose broth (pH 6.8) is boiled to expel dissolved gases; cool and inoculate with pus from an acne lesion. Add penicillin so that the broth contains a concentration twice as great as had previously been shown to inhibit staphylococcal growth. Run hot sterile Vaseline on to the surface to a depth of $\frac{1}{4}$ to $\frac{1}{2}$ in. to exclude air, and incubate at 37°. After incubation for 60 hours acne bacilli are perceptible and there is a copious growth in 4 days. This procedure enables autogenous vaccines of the acne bacillus to be prepared in 3 to 4 days after the material has been collected.—S. Craddock, *Lancet*, i/1942, 558.

The name *Bottle Bacillus* was formerly used as a synonym for acne bacillus, but the work of J. M. H. MacLeod and G. B. Dowling (*Proc. R. Soc. Med., Sect. Dermatol.*, 1928) showed that the two are very different organisms. The acne bacillus is a true bacillus present in lesions of acne, and it may possibly be the cause, but, according to MacLeod, it is doubtful whether this has been definitely proved.

The *Bottle Bacillus* these workers have definitely found to be pathogenic and the cause of seborrhoeic dermatitis. It is a yeast-like organism belonging to the group of the *fungi imperfecti*, and is related to *monilia*. The name should be discontinued and replaced by spore of *Malassez*, or *Pityrosporon Malassezii*. It can be stained by the Giemsa method and is pleomorphic, the flask-shape being characteristic. Average size 3 to 7 μ by 2 to 6 μ . Grows freely on maltose agar at 25°, peptone broth with 1% oleic acid and 1% glucose added. It is almost universally present on the human scalp.

Actinomycosis. A parasitic disease, due to the "ray fungus," *Streptothrix actinomyces*, first observed in cattle (wooden tongue), characterised by chronic inflammation, with or without suppuration, frequently resulting in formation of granulation tumours, especially about the jaws.

To identify the fungus. 1. Place specimen, pus or sputum, in a flat glass dish on a black surface. Remove the characteristic yellowish particles if found, and carefully tease out on a microslide or cover-glass. 2. Fix film over the flame. *Stain by the Gram-Eosin method:* Cover the film with alcohol for $\frac{1}{2}$ minute, then for 10 minutes with aniline gentian violet (1 part of concentrated alcoholic gentian violet and 9 parts of saturated aqueous aniline). Stain with Gram's iodine solution (iodine, 1 g.; potassium iodide, 2 g.; water, 300 ml.) for 3 minutes, decolorise in alcohol, wash in water and counterstain with eosin (5% aqueous solution).

The violet-stained mycelium of the fungus, will be seen as tangled webs or scattered branching filaments, on a pink ground (leucocytes, epithelia, etc.), with a $\frac{1}{4}$ -inch or even $\frac{3}{8}$ -inch objective.

The pus from an abscess is thin, greenish yellow, containing small clumps, about the size of a pin's head (the so-called "sulphur granules") composed of the streptothrix.

The "rays" may be observed without staining, but the stained specimens are confirmatory and are valuable for reference.

While in some text-books actinomycosis is supposed to be caused by two different organisms, *Actinomyces hominis* and *A. bovis*, the former an acid-fast, aerobic streptothrix commonly found in grasses, and the latter a non-acid-fast anaerobic streptothrix which has not been found outside man or animals, modern workers ascribe the disease solely to the latter, which was found in 29 consecutive cases seen in the London Hospital over 5½ years. *A. bovis* has been found in the tonsils of healthy persons, and in carious teeth, and the view is now held that infection takes place from organisms in the alimentary tract. The infection may be initiated by injury produced by foreign bodies (e.g. by chewing a straw) but the association of foreign bodies with the disease has been overstressed. *A. bovis* is always associated with other bacteria (e.g., the colon bacillus) and although in many cases the infection appears to be pure it will be found that a minute bacillus, *B. actinomycetum*, is also always present and constitutes the bulk of the gram-negative material observed in sections of the actinomycotic granule, and their constant presence is important as showing how the infection can be initiated.

Clinically, the infections can be divided into two main groups: the superficial group, including the cervico-facial variety and subcutaneous infections of the limbs, and the deep group, including the thoracic and abdominal cases. Prognosis depends on the anatomical site of the lesion—if near the surface, allowing ready discharge of its granular and pustular content, the patient recovers, but if deeply placed free drainage is not easily established and the patient may succumb from extension of the disease or from amyloid disease. In cervico-facial cases, before the formation of pus, trismus is the most important sign. Early diagnosis may be made by tilting a test-tube of actinomycotic pus, when the granules are visible to the naked eye as greenish-grey specks adhering to the glass. Where cases are allowed to suggest the disease by their chronicity the disease has usually spread beyond human aid.—R. Bates, *Lancet*, i/1933, 571.

True primary actinomycosis is rare; the presence of *B. actinomycetum comitans* is diagnostic. The relationship of this organism to *A. bovis* is not clear. No relationship established between "vegetable trauma" and the disease.—R. Klaber, *Brit. J. Derm.*, 1934, 12.

The pathogenic aerobic organisms of the actinomyces group: a description in cultural and morphological detail of 25 species, 15 of which are entirely new.—*Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 203, 1935.

Actinomycosis of the central nervous system is rare; 85 references were collected by Cope who suggests that spread to the central nervous system may take place in three ways: (1) An isolated lesion in the brain, often in the region of the pituitary body and fornix, by spread from the naso-pharynx along the perineural sheath of the olfactory nerve, (2) metastatic spread via the blood stream from the lungs; (3) direct spread from upper or lower jaw via connective tissue to foramina at base of skull.—Z. Cope, *Actinomycosis*, 1938.

Two cases of direct spread to central nervous system from cervico-facial region.—Eckhoff, *Lancet*, i/1941, 7.

More than 100 species of actinomyces, more or less pathogenic to man, have been recorded. Knowledge of the disease and organism is chiefly limited to *A. bovis* of Wolff and Israel. A case of actinomycosis of lower jaw, after many months of investigation, claimed to be associated with a new species, *A. moormani*, which probably occurs in the mouth as a saprophyte.—G. C. H. Franklin, *Ann. int. med.*, 1940, 13, 1205.

Treatment of actinomycosis in cases seen early enough is complete surgical excision of the lesion. This is rarely possible and in more advanced cases abscesses must be opened and sinuses curetted to promote drainage. X-ray

therapy and radium in carefully controlled dosage have been reported to give good results. Sometimes injection of 4% formaldehyde into the lesion, under anaesthesia, is of value. Chiefly reliance is placed on large doses of potassium iodide (30-90 gr., *t.d.s.*) over a long period. Vaccine therapy has produced excellent results in some cases.

Actinomycosis, 23 cases. Promoted efficient drainage and curetted with a dry gauze swab. Vaccine therapy employed.—L. Colebrook, *Lancet*, i/1921, 893.

Five cases successfully treated with thymol, 1 to 2 g. in capsules daily or on alternate days, combined with local injections of a 10 to 20% solution of thymol in olive oil once daily. The capsules of thymol should be taken on an empty stomach and followed by a glass of milk to minimise gastric irritation.—H. B. Myers, *J. Amer. med. Ass.*, i/1937, 1875.

Ankylostomiasis. *Syn.* HOOKWORM DISEASE. The worms producing this disease (*Ankylostoma duodenale* or *Necator Americanus*) are about $\frac{1}{4}$ inch long and of a pinkish-white colour when alive, but grey when dead and reddish-brown when gorged with blood. Their habitat is the small intestine of man, infection taking place either through the mouth or through the skin, and adult worms may remain alive in the intestine for as long as six years. They attach themselves to the mucous membrane, and no fewer than 1000 of them have been obtained from one patient. The eggs pass away from the patient—as many as 8,000,000 have been passed by a sufferer in a single day—and the small worm escapes from the egg and makes for moist earth or water. Mines afford an excellent hatching place for the young larvæ, which enter the skin of man often through the bare feet, producing vesicles or pustules (“ground-itch”) which heal in a week or so. The parasites pass in the circulation to the heart and lungs. They burrow into the bronchi and are carried with mucus up the trachea and pass down the œsophagus to the small intestine. Hygienic and sanitary measures are necessary to stamp out the disease, together with the discovery and treatment of carriers.

The symptoms vary according to the number of worms present but in the main the symptomatology is that of secondary anæmia; it has been stated that 500 worms must be present for six months to produce effects on the host. In severe cases there is often a ravenous appetite and a craving for earth.

In heavy infections the symptoms are largely related to the anæmia which is associated with a low colour index and an increased blood volume. The really severe case typically shows a tallow-yellow discoloration of the *ala nasi* and forehead, a dry earthy-coloured skin, pallor of the mucous membranes, mental sluggishness, dilated stomach and epigastric tenderness. Dyspnoea, cough, palpitation, pulsating cervical veins and hæmic murmurs are common; retinal hæmorrhage, œdema of the feet and serous effusions may occur.—G. C. Low and N. H. Fairley, *Price's Textbook of the Practice of Medicine*, 1941.

Hookworms take blood, and so iron, from those who are harbouring them and this is the first step on the road to hookworm anæmia; this loss has to be overcome by giving to the blood-making marrow iron from the food or from stores in the body, and this is the second step. When this loss has gone so far that serious anæmia is present, and when iron has then been given without de-worming the anæmia has become less. No cases have been observed in which the hæmoglobin has become normal under iron treatment so long as the worms have still been present in the blood. In the treatment of hookworm anæmia the first step has then to be the same as for hookworm infection, that is, de-worming, the second is the putting back of iron into the body.—Clayton Lane, *Trop. Dis. Bull.*, 1937, 12.

Treatment. Treatment consists in the administration of anthelmintics for the de-worming of the patient followed by the use of iron, and possibly of blood transfusions, to combat the anaemia. (*For details of anthelmintics, see Vol. I.*)

Iron and blood transfusions used for treatment of anaemia due to ankylostomiasis. After iron therapy, the transfusions gave a good response. The blood and bone marrow showed picture similar to that of hypochromic idiopathic anaemia.—Azmy and A. F. Zanaty, *J. trop. Med. (Hyg.)*, 1939, 42, 263.

Nervous complications. Symptoms of subacute degeneration of cord, without achlorhydria or pernicious anaemia, but with marked secondary anaemia due to *Ankylostoma duodenale*. Recovery in two cases after massive doses of iron, vitamin B and liver.—H. Hoff and J. A. Shaby, *J. trop. Med. (Hyg.)*, 1939, 42, 360.

Anthrax is primarily a disease of animals (sheep and cattle) caused by infection with *Bacillus anthracis*. If an animal die suspected of the disease the mode of examination is to cut off the ear and submit the blood from the same to bacteriological examination. The organism does not spore in the body of the animal, but if the air gain access, as in the case of an ordinary post-mortem investigation, the organism spores rapidly and hence becomes a grave source of danger.

The organism is non-motile and almost invariably occurs as long filaments, particularly in broth cultures. It grows on all the ordinary media both at room and body temperature, and produces in gelatin "stab" cultures typical "inverted fir trees" appearance. By growing at 42° a non-sporing form can be produced, which is the mode of attenuation for the immunisation of animals, as introduced by Pasteur. The spores retain their vitality and pathogenicity for years in dry conditions; they are very resistant to dry heat and withstand boiling for 5 minutes.

Staining of the blood may be conducted by Gram's method (counterstaining with eosin), also by alkaline methylene blue. The organism is gram-positive.

In man, infection is almost confined to workers in hides, hairs and wools, though it occurs occasionally in butchers. It is rarely caused by infected shaving brushes.

Treatment. The disease in man is treated by Sclavo's Serum or other anti-anthrax serum, or by neoarsphenamine (*see Vol. I*).

Of 9 cases treated with neoarsphenamine intravenously or sulpharsphenamine intramuscularly, 7 recovered; usually one injection of 0.6 g. was sufficient.—F. W. Gilbert, *Lancet*, ii/1935, 1283.

Ninety-three cases of cutaneous anthrax treated without excision of the lesion. In this country an arsenical should be regarded solely as an adjuvant to serum and only in the mildest cases, or where serum is not available, is its use alone to be recommended.—A. E. Hodgson, *Lancet*, i/1941, 811.

Bacterial Food Poisoning. By virtue of Section 7 of the London County Council (General Powers) Act, 1932, *food poisoning is a compulsorily notifiable disease in London*. A registered medical practitioner who suspects or becomes aware that a patient is suffering from food poisoning must forthwith notify the district Medical Officer of Health on a certificate stating: (a) the patient's full name, age and sex; (b) postal address of the house or premises where the patient is; (c) particulars of the food poisoning; (d) a statement whether the case is in the

notifying practitioner's private practice or in his practice as Medical Officer of a public body or institution. Failure to notify involves liability to a penalty not exceeding 40s.

The specimens which should be provided for the elucidation of an outbreak of food poisoning are, in order of importance, (1) the fæces of the sufferers during the acute stage; vomited matter is usually less instructive; and (2) portions of the actual food or drink on which suspicion can be thrown. In selecting the suspicious food or foods it is well to remember that milk, eggs and made-up meat dishes are at least as liable to have been responsible as more "suspect" articles, canned meats, for example. In outbreaks which have become known only after convalescence of the sufferers is established, the examination of their blood (by tests for agglutination of bacteria of the food poisoning group) may give useful information as to the nature of the infection; the blood, preferably drawn 8 to 10 days after onset of the illness, should be provided in at least 1 ml. quantity, since tests may have to be applied to a considerable number of different strains of the specified bacteria.

Food poisoning outbreaks of bacterial origin—chiefly caused by flesh foods—may be divided into three classes; (1) those due to the ingestion of food contaminated with living organisms of the Gaertner group bacilli (*Salmonellas*) which are responsible for the great majority of the large outbreaks; (2) cases of botulism—a small group due to *Cl. botulinum* (see *Botulism*, p. 880); (3) those due to the irritative action of toxic substances preformed in food or drink by the action of bacteria such as *Staphylococcus aureus*, *Staphylococcus albus*, *Streptococcus viridans*, *Streptococcus faecalis* and occasionally *B. coli* or *B. proteus*.

Gaertner Group Bacilli (*Salmonellas*). With the exception of *B. paratyphosus* A, B and C, practically all the *Salmonella* group of bacteria are capable of producing acute infective gastro-enteritis in man. The bacteria of this group resemble one another very closely in morphological and cultural characters; serologically they can be differentiated by agglutinin-absorption tests. The commonest food-poisoning *Salmonella* is *B. ærtrycke* (originally *Bacterium typhi-murium*), the usual host of which is the mouse. *B. enteritidis* (Gaertner) which occurs in natural infections in rats is generally considered the classical type member of the group. Other members are *B. suipestifer*, and many other varieties designated by specific names such as "Newport," "Stanley," "Thompson," "Dublin," *B. morbificans bovis*, etc. They possess the following characteristics—short, sporeless bacilli, with rounded ends; motile with numerous long lateral flagella; Gram-negative, grow readily on ordinary nutrient media; readily killed by heat, antiseptics, drying or sunlight; can survive for long periods at 0° or below; do not produce indole, some produce H₂S; ferment dextrose and mannitol (with formation of acid and gas); do not ferment lactose.

Pathogenic members of this group, including *B. enteritidis* and *B. suispestifer* have the property of producing in the animal body toxins which are remarkably heat-resisting. At one time the organisms of this group were confused with *B. paratyphosus* B, with which they have a close serological relationship, but *B. paratyphosus* B is associated with paratyphoid fever and probably never produces food poisoning.

The three common ways in which food becomes infected by *Salmonellas* are: (1) by the use of the flesh of animals (ducks, bovines, pigs, etc.), insufficiently cooked, for foods; (2) by contamination of food with excreta containing the specific bacteria (e.g., by mice or rats); (3) by the handling of food by human beings infected from one or other of these animal sources and who may discharge the infection in their faeces (two outbreaks during 1935 were traced to this source).

Bacteriological Diagnosis of Salmonella Poisoning. Make cultures from the stools (or suspected articles of food) on plates of MacConkey's medium (bile-salt-lactose neutral-red agar). The *Salmonella* colony appears as a pale translucent disc; coli groups appear as opaque red colonies (lactose fermenters). Test fermentation reactions to sugars and agglutination reactions. To distinguish *B. paratyphosus* B from food poisoning organisms, e.g., *B. aertrycke*, use Pesch's medium containing ammonium chloride and rhamnose. *B. aertrycke* grows on this, but *B. paratyphosus* B does not. Sodium *d*-tartrate 1% in peptone water is also useful. *B. aertrycke* and various other *Salmonellas* use this salt, but *B. paratyphosus* B does not. After incubation add solution of lead acetate to the culture; the occurrence of a heavy precipitate shows that the tartrate has not been used; no precipitate (or slight precipitate due to carbonate) shows utilisation.—Muir and Ritchie.

Differentiation of Salmonella Food Poisoning Bacteria. The identification of the types by absorption-agglutination tests depends upon the fact that each type produces a heat-stable O antigen contained in the bacillary body (somatic antigen) and a heat-labile H antigen (flagellar antigen). The latter contains group-specific and type-specific components. Some organisms in the group are "diphasic," the group antigen being carried by some bacteria, and the type-specific antigen being carried by others. The determination of the O antigen affords a method of assigning an organism to a sub-group. For this purpose O-agglutinating anti-sera for representatives of these sub-groups are required (*B. paratyphosus* A, *B. paratyphosus* B, *B. suispestifer* (monophasic) and Newport type, *B. enteritidis*, etc.). Direct agglutination with one of these points to the type of O antigen; confirm by absorption-agglutination. To find the phase of a diphasic organism an H-agglutinating anti-serum for the monophasic *B. suispestifer* (group phase only) is tested with a formalised suspension of the unknown strain. Large flake agglutination shows organism probably in group phase and specific phase derivative must be separated from it. This can often be done by plating to obtain separate colonies. Loopfuls from each of a number of colonies are separately emulsified on a slide in a drop of anti-serum (in low dilution) to *B. suispestifer* (monophasic). Colonies which do not agglutinate are presumably in the specific phase. Formalised subcultures from these colonies are tested for agglutination with pure specific-phase H anti-sera for each member of the sub-group to which the organism has been assigned.—Muir and Ritchie (Classification of *Salmonellas*, Sub-Committee of International Society of Microbiology), *J. Hyg., Camb.*, 1934, 34, 333.

Staphylococcal Food Poisoning. *Staphylococcus aureus* is considered to be the most important of the bacteria which produce gastro-enteritis by the irritative action of substances formed in the food by bacterial action. The bacteria need not necessarily be alive at the time of ingestion. The toxins which they produce in food are not strictly analogous to those of the tetanus

or diphtheria bacilli; they withstand boiling without much loss of toxicity. Some workers consider that they may be the autolysed bodies of bacteria that have lived and died in the food. The activity of *Staphylococcus aureus* as an enterotoxin-producer is very variable; some strains produce no toxin in either ordinary foods or laboratory media; other strains produce substances which are highly irritant to the gastro-intestinal mucosa. The property of producing gastro-toxic substances is not related to the presence in large amount of either staphylococcal hæmolyisin or the dermo-necrotic toxin (see p. 954).

Foods liable to contain toxin produced by staphylococci or other bacteria are canned and potted meat or fish, meat or fish pastes; milk; foods containing soluble starch, especially artificial "creams."

In twenty outbreaks of food poisoning apparently due to staphylococci, affecting 1028 individuals, all of which followed consumption of "ready to eat" ham or tongue, two deaths occurred but neither was attributed directly to food poisoning. In many of the outbreaks there was little evidence to indicate the source of the staphylococci, or when in the process of manufacture or distribution contamination occurred. Study of 37 strains of staphylococci isolated from food involved in 11 outbreaks demonstrated that the majority had lost their power to produce enterotoxin and to give a positive Stone reaction during storage on laboratory media. Enterotoxic and non-enterotoxic strains differ in their agglutination reactions to normal horse serum.—G. G. Slocum and B. A. Linden, *Amer. J. publ. Hlth*, 1939, 29, 1326.

Report of 31 cases of staphylococcus food poisoning following consumption of food prepared with hollandaise sauce which was served hot—showing the relative stability of staphylococcus toxin to heat. In the severe cases there was persistent vomiting, cramps, profuse diarrhoea (in one case blood-tinged) and alarming prostration. The acute enteritis subsided in 8 hours and subsequent recovery was uneventful. One case was so severe that intravenous sodium chloride and dextrose were thought necessary. Cultures of a specimen of the sauce gave hæmolytic staphylococci, both aureus and albus.—per *J. Amer. med. Ass.*, 1/1939, 1362.

A case of acute bacterial food poisoning, due to hæmolytic *Staphylococcus aureus*, ingested in soup, which had been contaminated a week previously by a suppurative lesion of the patient's thumb. Within two or three hours after taking the contaminated soup the patient became acutely ill with vomiting and diarrhoea. The symptoms of gastro-enteritis increased, and death occurred 22 hours after the onset. Hæmolytic and coagulase-positive *Staphylococcus aureus* was obtained in cultures from the stomach and from the inside of the empty soup tin.—G. C. Dorling, *Lancet*, 1/1942, 382.

In staphylococcal food poisoning the incubation period is short (2 to 4 hours) compared with 6 to 12 in Salmonella food poisoning. From the staphylococci isolated from this type of food poisoning may be isolated a bacteria-free filtrate, which, when swallowed, reproduces the symptoms of an acute gastro-enteritis, whereas bacteria-free filtrates from Salmonella cultures have been fed in large amounts to humans without producing any symptoms.—E. M. Wade, *Nat. med. Bull., Wash.*, 1938, 36, 306.

Bejel. *Syn.* MUCOCUTANEOUS SYPHILIS, NON-VENEREAL SYPHILIS. Bejel is the name given by the Arabs to an endemic form of syphilis, an afebrile malady with little effect upon the constitution, affecting 90% of the Bedouin village population along the banks of the Euphrates river in Syria, and usually contracted by or from children. It exhibits mucocutaneous and periosteal lesions in the early cases, and, after a latent period of varying length, late lesions appear in the form of mucocutaneous relapse or gummata of skin and nasal and long bones. Bejel is

uniformly accompanied by positive precipitation and complement fixation reactions, and spirochætes indistinguishable from *Treponema pallidum* are constantly found in the open lesions of early eruption and late relapse.

The child-syphilis produces two conditions which have hitherto been thought to be characteristic of yaws. The one is thickening and fissuring of the skin of the soles of the feet, at the point which bears the weight of the body, and cracking of the heel. The second is a patchy pigmentation of the skin of the hands. —E. H. Hudson, *Ann. trop. Med. Parasit.*, 1936, 30, 3.

Details are given of 1000 cases of bejel treated with daily intramuscular injections of bismuth in the form of sodium bismuth tartrate and bismuth salicylate, and receiving an average total of 75 cg. of the metal. Improvement was usually immediate, and the healing effect was prolonged beyond the treatment period. 49% of the patients were improved, and 28% were healed. The result of treatment was not known in 13%, and 10% were not changed.

Bismuth is less toxic and more effective than mercury; it is more suitable to the conditions of bejel than the arsenicals, and is also easier of administration, less hazardous, and much cheaper. —E. H. Hudson and S. S. Crosley, *J. trop. Med. (Hyg.)*, 1936, 249.

The apparent absence in bejel of the initial lesion known as chancre in venereal syphilis is noteworthy. —E. H. Hudson, *New Engl. J. Med.*, ii/1936, 392.

The view is advanced that bejel is the archetype of syphilis. It is suggested that the similar immunology of bejel and yaws stem from their non-venereal epidemiology. It may be that the urban portion of the human race, by imposing a venereal epidemiology upon the spirochæte, has changed the clinical course and the pathology of syphilis, producing in civilised countries a far more serious disease than the original syphilis of rural children. —E. H. Hudson, *Trans. R. Soc. trop. Med. Hyg.*, 1937, June 9.

Among the Bedouin in Kawait (Arabia) and its outlying desert the word "belesh" is used in much the same way as the Euphrates Arabs use "bejel," and, as on the Euphrates, the disease usually begins in the mouth. Of the efficacy of bismuth there is no longer the least doubt, and Sobita, or sodium bismuth tartrate, is the preparation of choice. Pain following the injection may be avoided by giving it in a solution of 50% glycerin in distilled water and advising the patient to rest for 24 hours after injection. —C. S. G. Mylrea, *Trans. R. Soc. trop. Med. Hyg.*, 1937, July, 259.

Throughout much of the Sudan "bagl" ("g" usually soft) is a well-known disease, and there is no doubt that "bejel" and "bagl" are transliterations from the same Arabic word. In the Sudan, however, "bagl" is a colloquial word with the sole meaning of gonorrhœa. —H. Richards, *Trans. R. Soc. trop. Med. Hyg.*, 1937, Nov., 369.

Blackwater Fever. *Syn.* MELANURIC FEVER; MALARIAL HÆMOGLOBINURIA. Blackwater fever is generally characterised by the occurrence of severe rigors at the onset with bilious vomiting, jaundice and hæmoglobinuria. It is not a disease *per se* but is associated with repeated attacks of, or continuous infection with, sub-tertian malaria. An association has been noted between the quinine treatment of the malaria and the onset of hæmoglobinuria, but quinine is not the primary cause of attacks of blackwater fever since the disease occurs in patients treated with mepacrine and pamaquin. The hæmoglobinuria of blackwater fever resembles that associated with primary hæmolytic anæmias and independent studies of these diseases have shown a close relationship. Hæmoglobinuria results when sudden intravascular lysis of red blood cells releases hæmoglobin into the plasma in amounts sufficient to exceed the renal threshold, i.e., when the free hæmoglobin in the plasma exceeds 100 to 140 mg. per 100 ml. This conclusion is based on the effects of injection of solutions of hæmoglobin into

the blood stream reported by N. H. Fairley (*Brit. med. J.*, ii, 1940, 213) and D. R. Gilligan *et al.* (*J. clin. Invest.*, 1941, 177).

When quinine was given to rabbits, the red blood-cells became less resistant *in vitro* to the lytic action of substances such as sodium taurocholate, but quinine alone in concentrations similar to those in the blood did not cause hæmolysis. The quinine was thought to accelerate the action of intravascular lysins.—E. Ponder and I. C. Abels, *Proc. Soc. exp. Biol.*, N.Y., 1936, 34, 162.

The red blood-cells of malarial patients were more sensitive to hæmolysis by bile when quinine was given.—G. Mev, D. Birnbaum and I. J. Kligler, *Trans. R. Soc. trop. Med. Hyg.*, 1941, 373.

The relationship between the activity of the malarial parasites and the accelerating effect of the quinine was observed in a patient with mild blackwater fever; when the malaria was inactive hæmoglobinuria was not brought on by quinine.—N. H. Fairley and F. Murgatroyd, *Trans. R. Soc. trop. Med. Hyg.*, 1940, 34, 187.

The plasma of blackwater fever patients contains a pigment—methæmalbumin—which is also present in the plasma after any hæmolytic event.—N. H. Fairley, *Brit. med. J.*, ii, 1940, 213.

Chronic malaria leads to hypertrophy of the reticulo-endothelial cells and an increase in their normal functions including erythrocyte destruction; the formation of an intracellular lysin, or enzyme, normally responsible for the destruction of aged red blood-cells is increased and it is liberated into the circulation when it becomes fixed at once to the red blood-cells. Sudden liberation in large amounts leads to the hæmoglobinæmia and consequent hæmoglobinuria of blackwater fever.—N. H. Fairley, *Trans. R. Soc. trop. Med. Hyg.*, 1940, 34, 173; *Lancet*, i, 1941, 667.

25 ml. of blood from a patient with typical black acid urine, containing oxyhæmoglobin, and methæmoglobin, and with methæmalbumin in the blood plasma, were injected into a normal healthy man. Eleven days later, although he had been kept strictly away from possible outside infection, the man developed a typical malarial attack, and *Plasmodium falciparum* rings were found in six films of his blood, but he did not develop hæmoglobinuria. In a previous series of 106 persons who received intramuscular injections of blood from blackwater fever patients, none showed hæmoglobinuria, although several developed malaria. Blood transfusions given to the blackwater fever patient from three different normal donors did not reduce the hæmolysis, suggesting that normal cells are just as liable to hemolyse in a blackwater fever patient's blood stream as are the patient's own cells. It is concluded that there is no specific hæmolyzing strain of malarial parasite and that a circulating hæmolyisin is the active factor that produces the intense hæmolysis of blackwater fever.—H. K. Foy and A. Mounjidis, *Trans. R. Soc. trop. Med. Hyg.*, 1941, 35, 119.

Prophylaxis. Malaria prophylaxis is also the method for the prevention of blackwater fever, i.e., quinine should be taken regularly and in sufficient doses, and on leaving a malarious district quinine or mepacrine should be taken continuously for at least six months.

Treatment. Absolute rest in bed is essential; the patient should never be moved from the place where he is taken ill. Plenty of fluids should be given and large doses of alkalis (e.g., sodium bicarbonate) administered either by mouth or intravenously. The ordinary antipyretics should not be used; frequent sponging is usually sufficient. Blood transfusion has proved of value in many cases.

Ten cases successfully treated by Atebrin and Campolon injections. Campolon, besides being a powerful stimulant of the hematopoietic system exerts a specific action upon the liver function.—H. E. Naumann, per *Trop. Dis. Bull.*, 1936, 523. Two cases successfully treated with large doses of Campolon alone.—I. H. Otto, *ibid.* Of 10 cases treated with Atebrin and Campolon only 1 died as compared with 7 deaths among the 13 cases treated by other methods previously.—A. Corman, *ibid.*, 524.

Blood transfusion has been systematically employed in the treatment of blackwater fever in Southern Rhodesia over a period of 5 years and it is felt that it is an important life-saving measure. It must be instituted, however, in the early stages of the disease and the transfusions must be repeated until there is hematological evidence of active erythropoiesis. While slavish adherence to actual red cell counts as a guide to transfusion is not advocated, the broad

principle may be adopted that a count of 2,500,000 to 3,000,000 with a reticulocyte count of 5% or less warrants its employment, while a rapidly increasing reticulocytosis in association with an improvement in the count serves as an indication that it may be discontinued. The blood should be given slowly and the total volume of citrated blood administered at any one time varies between 350 and 450 ml.—W. K. Blackie, *Lancet*, ii/1937, 1124.

In the mild type of blackwater fever recourse to blood transfusion is unnecessary; blood transfusion is definitely contra-indicated in the anuric cases, in fact, it appears to aggravate the condition and has not been found to improve the urinary secretion. In the toxic polyuric type of case, blood transfusion is of distinct value. There is, however, no evidence to show that blood transfusion has any effect in reducing the period of hæmolytic, but it counteracts the tendency to development of a severe grade of anæmia.—P. Manson-Bahr, *Med. Annu.*, 1939, 61.

Transfusion, even in moribund cases with red cell counts as low as 800,000 per c.mm., is a life-saving measure, provided that renal function is being maintained.—H. Foy *et al.*, *Trans. R. Soc. trop. Med. Hyg.*, 1941, 35, 119.

Blastomycosis. Two forms of the disease are recognised; (a) the cutaneous form of blastomycetic dermatitis of Gilchrist, which commonly affects the face, back of the hand and front of the leg. The causative organism is *Cryptococcus gilchristi*, which is spherical, resembling a yeast cell, and has a doubly-refractile capsule; there are no endospores and reproduction is by budding. In artificial media it forms long mycelial fragments, but these are not seen in the parasitic phase; (b) the systemic form (**California disease**) caused by *Coccidioides immitis*, which is larger than the cryptococcus and reproduces chiefly by endosporulation. It has a doubly-refractile capsule containing many spores; hyphæ are formed only on artificial culture media, not in pathological lesions. The organism commonly gains entrance to the body via the respiratory tract. Any organ except the intestines may be affected but the commonest is the lung. The fungus may be detected by adding a few drops of a solution of sodium hydroxide to the pus and examining it unstained. The disease is mostly confined to the U.S.A. and is rare elsewhere though some cases have been reported from India. (For a description of blastomycosis and blastomycosis-like infections see L. M. Smith, *J. Amer. med. Ass.*, ii/1941, 200.)

Treatment. Large doses of iodides (20–60 gr., *t.i.d.*) are said to be specific for the cutaneous form. The systemic form is almost invariably fatal. Good results from intravenous injections on alternate days of 2 to 8 ml. of 1% solution of tartar emetic and $\frac{1}{2}$ skin unit of unfiltered X rays at intervals of from 10 to 14 days, the injections being spread over 3 to 4 months.—C. C. Tomlinson and P. Bancroft, *J. Amer. med. Ass.*, i/1934, 36.

Botulism. An acute variety of food poisoning caused by *Clostridium botulinum* (*B. botulinus*) of which there are three types A, B and C. The exotoxin, which is present in infected food, acts on the nervous system. Any animal or vegetable food may be infected, but the commonest examples are canned or potted vegetables (especially home-canned, when sterilisation has not been effectively carried out); less often meat eaten uncooked. Outbreaks have been traced to potted meat paste prepared from wild duck (Loch Maree outbreak, 1922—*Brit. med. J.*, i/1923, 279); "Vegetable brawn" (London outbreak, 1935—*Rep. med. Offr Minist. Hlth, Lond.*, 1935, 157); canned spinach, runner-beans,

olives; sausage and smoked ham eaten raw (Germany and elsewhere in Europe).

Clostridium botulinum is a normal inhabitant of soil; it is a Gram-positive, anaerobic, spore-forming organism about 4-6 μ in length; in straight rods with rounded ends and 20-30 lateral flagella; the spores are oval and subterminal; growth occurs in all the usual media anaerobically. There are three types, A, B and C. All types ferment glucose but not lactose or sucrose; types A and B are proteolytic, type C is not. All types produce a soluble exotoxin in nutrient media; type A toxin is the most powerful and is responsible for most fatal cases of botulism; type B toxin is less toxic and no cases due to it have yet been reported in Great Britain; type C toxin is still less dangerous and so far no human case due to it has been described. The optimum temperature for toxin production is 37°. The spores are highly resistant to heat and will survive two hours moist heat at 100°.

The organism acts on the peripheral nervous system; it is not a gastrointestinal irritant. The most characteristic symptoms are the following: disturbance of vision appears early, including dimness of vision, mydriasis, diplopia, and loss of reflex to light stimulation; later there is complete loss of accommodation. Another group consists of a feeling of throat constriction and difficulty in swallowing and talking. There may be complete paralysis of the pharyngeal muscles. Eventually there is complete aphonia. General muscular weakness is a characteristic feature, while there may be inco-ordination of muscle movement. Inhibition of the secretions is characteristic, especially dry mouth and pharynx. While in the initial stages diarrhoea may occur, the characteristic feature is marked constipation. There is no rise in temperature, and sensory disturbances are entirely absent.—Sir W. Savage, *Brit. med. J.*, ii/1942, 585.

Botulinus Antitoxin (Serum Antibotulinum, E.P.C.). There is no official standard of potency. A supply of botulinus antitoxin is maintained under the control of medical officers of health at sixteen provincial centres in Great Britain and is also available at the Ministry of Health, Whitehall, London, S.W., at any hour of the day or night. No antitoxin should be used containing less than 100 units per ml. (1 unit is defined as the amount of serum required to neutralise 100 minimum lethal doses of toxin for guinea-pigs). **Dosage:** *Prophylactic* for persons who have consumed infected food but not yet developed symptoms—10 ml. intramuscularly.

Treatment. Prompt administration of type-specific antitoxin is essential to prevent the fixation of toxin by the tissues. 10 ml. of antitoxin mixed with 100 ml. of normal saline may be given intravenously by gravity, or the antitoxin may be given with a continuous drip glucose-saline. There is no limit to the total amount of antitoxin which may be given; the 10 ml. dose should be repeated hourly until the paralysis ceases to progress. The possibility of serum reaction should be ignored in view of the gravity of the condition. Respiratory paralysis should be treated with some form of mechanical respirator.—W. M. Scott, *Brit. Encycl. med. Pract.*, 2, 589.

The demonstration of botulinus toxin in the blood, apart from the demonstration of *Cl. botulinum*, should be of value in the diagnosis of the disease. It is generally believed that the amount of botulinus toxin necessary to produce symptoms in the patient is so minute as to preclude its detection in the blood, but Bergmar Insulander and Lindblad (*Acta med. Scandinav.*, 1935, 84, 496) demonstrated botulinus toxin in one case on the 7th day of illness and in another on the 10th day. The presence of botulinus toxin in the livers of three persons who died of botulism was demonstrated in an extract made by triturating 50 g. of liver with 50 ml. of normal saline solution. 1 ml. of this extract injected intraperitoneally into guinea-pigs, or 0.5 to 1 ml. similarly injected into mice caused death from experimental botulism. In one case blood collected from the patient before administration of antitoxin injected intraperitoneally into mice in doses of 0.5 ml. gave symptoms of experimental botulism.—H. J. Schreider and R. Fisk, *J. Amer. med. Ass.*, i/1939, 2299.

Differentiation of General Food-poisoning and Botulism. A comparison of the symptomatology, incubation period, treatment, mortality, and investigative procedure in the two types of food poisoning:—

| | General food poisoning | Botulism |
|-------------------------|--|---|
| Incubation period | Usually 3 to 8 hours; rarely over 12 | Usually 24 to 48 hours. |
| Symptomatology | Sudden onset; nausea, vomiting, abdominal pain, prostration, diarrhoea, and rise of temperature. | Delayed onset; marked muscular weakness; gastro-intestinal symptoms, rare; disturbances of vision with diplopia and blepharoptosis; loss of ability to swallow and talk; constipation, rapid pulse and subnormal temperature; rarely any pain; death from respiratory failure. |
| Case infectivity rate | High | Usually 100%. |
| Mortality | 0 to 1% | Over 60% |
| Investigative procedure | <ol style="list-style-type: none"> 1. Use incubation period for basis of determining the causative meal. 2. Always suspect freshly cooked or warmed-over foods. Preserved foods are rarely at fault. Foods are apparently good as to taste, appearance, odour and texture. 3. Bacteriologic examination of excreta of patients and the suspected food for the paratyphoid group and other organisms. Feeding of white mice and perhaps other laboratory animals with suspected food, both direct and by stomach tube. Likewise, injection and feeding of filtrates of bacterial organisms isolated from suspected food. 4. Bacteriologic and epidemiologic search for human carriers and possible contamination from animal sources, especially rats. Sanitary survey of source of suspected food, especially important where cream fillings or sauces are involved and the staphylococcus is the predominating organism. 5. Complications: Appendicitis, cholecystitis, persistent elevation of temperature (paratyphoid infection). | <ol style="list-style-type: none"> 1. Use incubation period for basis of determining the causative meal. 2. Always suspect preserved foods; likewise, meat products such as sausages. Spoilage of foods is noted in many instances. 3. Test of suspected food for toxin by animal inoculation; mice, guinea-pigs or rabbits. Test for type with specific antitoxin. Culture of suspected food for the presence of spores, particularly if food has been previously boiled. 4. Search for domestic animals, such as chickens with symptoms of limber-neck, for corroborative field and laboratory evidence. 5. Complications: Broncho-pneumonia. 6. Human outbreaks are usually due to Type A toxin. Botulinus antitoxin, specific type; absolute quiet; eliminative; and glucose solutions. |
| Treatment | Supportive and eliminative .. | |

Cerebrospinal Fever. An acute epidemic disease, characterised by profound disturbance of the central nervous system, indicated at the onset chiefly by shivering, intense headache or vertigo, or both, and persistent vomiting; subsequently by delirium, often violent, alternating with somnolence or a state of apathy or stupor; an acutely painful condition with spasm—sometimes tetanoid—of certain groups of muscles, especially the posterior muscles of the neck, occasioning retraction of the head and an increased sensitiveness of the surface of the body. Throughout the disease there is marked depression of the vital powers, not infrequently collapse, and during its course an eruption of vesicles, petechial or purpuric spots, or mottling of the skin, is apt to occur.

The chief predisposing causes are overcrowding, inadequate ventilation nasopharyngeal catarrh, the introduction of susceptible persons into a community, and the virulence of the organism. Late winter and spring form the meningococcal seasons in Europe, the disease being most prevalent in February and March. Children under ten are the most susceptible, adolescents and young adults next. Males seem to be twice as often affected as females. The introduction of sulphanilamide and sulphapyridine therapy has completely transformed a protracted and highly dangerous disease into a relatively minor malady. The mortality, which used to range from 25 to 98%, according to age, has fallen to 5% or less, irrespective of age.—*Med. Annu.*, 1941, 66.

Chronic meningococcal septicæmia, though not rare, is commoner when cerebrospinal fever is prevalent; it is probably often overlooked and regarded as subacute rheumatism, influenza or trench fever. 17 cases of chronic meningococcal septicæmia without meningitis were reported during an epidemic of cerebrospinal fever in the B.E.F. in France. Meningococcus isolated in only 3 cases. Clinical picture characteristic. Onset sudden and acute, with fever, severe headache, migratory pains in joints and muscles; a few days later a skin eruption appears, the commonest being pink or red macules, papules and nodules which may be painful and tender. Pyrexia 100°F. to 105°F. may persist intermittently for weeks or months if untreated; remarkable freedom from debility. Complications include bacterial endocarditis, nephritis and epididymitis. Terminates in 24 hours dramatically with sulphapyridine.—A. W. Stott and W. S. C. Copeman, *Lancet*, 1/1940, 1116.

Bacteriology. The meningococcus (*Diplococcus Intracellularis meningitidis*) responsible for the disease is a small coccus 0.8 to 1 μ in diameter and resembles the gonococcus, *M. catarrhalis*, *M. flavus*, etc., in morphology, being usually intracellular. It stains readily with basic aniline dyes but is non-staining by Gram's method. It ferments maltose and glucose, with production of acid, but has no action on sucrose or lactose.

Cultivation. The organism can be cultivated aerobically outside the body, but the conditions for its growth are somewhat restricted. Either agar with the addition of serum, ascitic fluid or blood, or boiled blood-digest agar is suitable. The optimum reaction is pH 7.5 and optimum temperature 37°; growth does not take place below 25° and even at 37° special precautions must be taken to prevent drying.

In the preparation of a medium for culture purposes most attention has been directed to the readiness and abundance with which growth takes place, but retention of virulence of the organism should also be a criterion of suitability. Murray and Ayrton found that retention of virulence depends upon substances contributed by the digest used as a basis and that the addition of an extract of polymorphonuclear leucocytes raises the virulence *in vitro*.—Muir and Ritchie.

In view of the fact that a large proportion of the patients will have received sulphonamide therapy before culture of cerebrospinal fluid or blood can be carried out, the successful recovery of the meningococcus cannot be so readily assured as it was before the introduction of this group of drugs. In order to overcome the bacteriostatic effect of the sulphonamide derivatives, the addition of p-aminobenzoic acid (5 mg. per 100 ml.) to the cerebrospinal fluid or the blood culture medium is recommended.—W.O. Memo. on Cerebrospinal Fever among Troops, 1942 (H.M.S.O.).

By the use of the following medium, growths of meningococci have been obtained with material which was negative on microscopical examination and also on attempts at cultivation on the usually employed media. One part stock nutrient agar is added to 6 parts of broth, and a 10% glucose solution is added to give a concentration of 0.1%. The medium is tubed in small tubes (10 cm. \times 1 cm.), 4.5 ml. being added to each tube and autoclaved. When sufficiently cool 0.5 ml. of human or rabbit serum is added sterily to each tube. The final reaction should be pH 7.2 to 7.4. The spinal fluid is centrifuged for 5 minutes at 2000 to 3000 r.p.m. and the sediment inoculated into the culture tubes the contents of which have been raised to body temperature. After 18 to 24 hours a disc of growth appears.—J. Gurevitch and I. J. Kligler, per *Bull. Hyg.*, 1936, 941.

The meningococcus possesses both "rough" and "smooth" strains. Rough cultures are likely to be less potent antigens for the production of serum than smooth cultures.—G. F. Petrie.

Agglutination Reactions. On the basis of direct agglutination tests Griffiths has classified meningococci into two groups (I and II) and this classification has now replaced the original classification into four types by Gordon (see Vol. I, p. 1035); Group I is more complex in antigenic structure than Group II and this may be associated with increased virulence. The serum of patients with meningococcal meningitis usually gives agglutination reactions to the meningococcus about the 4th day of the disease at a dilution of 1:50, rising later to 1:1000.

West's Swab for taking swabbings from the posterior wall of the pharynx consists of a wire in a bent glass tube and resembles Bellock's sound in glass instead of metal. These obviate the use of a tongue depressor. Inoculations are made *direct* on to Nasgar Medium and grown for 24 hours. Likely colonies can be cultivated on plates and incubated at 37° and at 23°. Colonies growing at 37° and not at 23° and having the requisite appearance are taken as meningococci. (N.B.—Whatever medium is used cultures must always be prepared immediately.)

Meningococcal Complement-Fixation Test. Technique used, that of the standard Wassermann reaction (No. 1 in *Spec. Rep. Ser. med. Res. Coun. Lond.*, No. 14), but instead of using a mixed suspension of local strains of meningococci all four types are included in known proportions. A saline suspension of pure cultures used containing in each ml. 90 million Type III, 60 million Type I, 50 million each of Types II and IV. Complement-fixation with meningococcal antigens appears in serum of at least 75% of cases of cerebrospinal fever, and a positive reaction in a case with meningeal symptoms is practically diagnostic of a meningococcal infection, though a negative reaction does not necessarily exclude this.—H. A. Cookson and J. E. Sinclair, *Lancet*, ii/1933, 634.

The polyvalent precipitin reaction with polyvalent antimeningococcal horse serum and the centrifuged spinal fluid is a reliable method of diagnosing meningococcal infections of the cerebrospinal system.—B. G. Macgrath, *Lancet*, i/1935, 545. Also *Lancet*, i/1934, 17.

Transmission. The old view concerning transmission was that the organism, by whatever way transferred, gained lodgment in the naso-pharynx and thence migrated to the base of the skull and the meninges. The present view is that infection takes place by direct contagion and that it is primarily a blood infection localising later in the central nervous system—indeed, it frequently remains confined entirely to the blood stream, not invading the central nervous system at all, and it is now usual to make blood cultures, as well as spinal fluid ones.

Treatment. The specific remedies are, at the present time, almost entirely confined to drugs of the sulphonamide series. The addition of specific serum-therapy to chemotherapy does not appear to influence the course of the disease. There are four sulphonamide derivatives the value of which in the treatment of cerebrospinal fever has been substantiated, namely, sulphanilamide, sulphapyridine, sulphathiazole and sulphadiazine and of these sulphadiazine would appear to be the most suitable and the least toxic (*cf.* Vol. I, p. 962); it has the added advantage of producing with the same dosage a more sustained higher average blood level than the other derivatives.—W.O. *Memo. on Cerebrospinal Fever among Troops*, 1942 (H.M.S.O.); see also *The Medical Use of Sulphonamides*, *Med. Res. Coun. War Memo. No. 10*, 1943.

Chagas' Disease. *Syn.* SOUTH AMERICAN TRYPANOSOMIASIS.

A disease occurring in parts of South America, and particularly in Brazil, due to infection with the trypanosome *Schizotrypanum cruzi*, transmitted by the bite of the reduviid bug, *Triatoma megista*. The trypanosomes are found in the peripheral blood only for a short time after infection, after which they assume a Leishmanial form within the cells of different organs where they undergo division and from time to time pass back into the peripheral circulation. The acute form of the disease is usually met with in infants under 1 year. In infants and young children the illness commences with a febrile disturbance, the temperature in severe cases rising to 104°F. A very common early sign is swelling of the eyelids and face; the œdema is firm, elastic and painless, and may be so marked that the eye cannot be opened. It is believed that the bugs bite the closed eyelids or the neighbouring regions of the face and the child inoculates the wound or the conjunctiva by rubbing in the infected excreta. In extreme cases the œdema may spread widely and involve the extremities and even the whole body. Associated with the œdema is an adenitis. There is progressive anæmia and the pulse is frequent, the tachycardia being independent of the temperature. The liver and spleen are enlarged. In severe cases nervous symptoms, such as irritability, convulsions, twitchings and clonic contractions have been described. The acute stage of the disease is of short duration. Among the severe cases which are met with, as a rule in very young children, a considerable proportion of deaths has been recorded. In cases surviving the acute stage the temperature usually returns to normal within a few weeks, and with the fall of temperature trypanosomes disappear from the blood and the œdema and other signs subside. There seems to be a *prima facie* case that American trypanosomiasis may be responsible for a good deal of the heart disease which is apparently so common in certain endemic areas in Brazil, Uruguay and the Argentine, and the cause of so many early deaths. The chronic cardiac form of the disease may be a sequel to an acute infection in infancy or the consequence of repeated infection in later life. If this should eventually prove to be the case then American trypanosomiasis will assume a pathological significance of the first magnitude.—Warrington Yorke, *Trop. Dis. Bull.*, 1937, 275.

Chagas who first described the disease in 1913, laid stress on the involvement of the thyroid gland, but this has since been the subject of much criticism and there is no evidence from experimental inoculations of animals that the trypanosomes especially involve the thyroid gland. A serological reaction for the diagnosis of the disease in cases in which trypanosomes cannot be found in the blood was devised in 1913 by Guerreiro and Machado. In this reaction (the **Machado Reaction**) extracts of the heart or spleen of infected puppies are used as antigen, but these extracts vary widely in their activity. A modification of the reaction by Kelser (*Amer. J. trop. Med.*, 1936, 16, 405) uses cultures of *S. cruzi* as antigen. If the specificity of this reaction can be established it may throw light on the chronic effects of this infection and enable the geographical distribution of the infection to be worked out.—*Lancet*, i/1940, 417.

The complement fixation test is of distinct value not only in identifying actual cases of Chagas' disease but in revealing the incidence of the infection. A

survey carried out in Panama indicates that the disease and the human carriers of the organisms are more common than has been supposed.—C. M. Johnson and R. A. Kelsner, *Amer. J. trop. Med.*, 1937, 385.

Chancroid. Chancroid ("soft sore") is ulceration of the external genitals caused by infection by Ducrey's bacillus (*H. ducreyi*), usually running an acute course and destructive of tissue. The causal organism is specific—it is an anærobic bacillus difficult to identify and culture. The incubation period is short, usually 1 to 3 days, but occasionally it may be a week or longer.

The disease is relatively uncommon in Great Britain, but is common in Europe, especially in Mediterranean ports, and in the East. In Britain, cases are not rare among sailors and those returning from the East. Males are affected more than females. There are usually several ulcers situated in the coronal sulcus or on the glans penis or on the inner surface of the prepuce. The appearance of several ulcers in succession is of value in distinguishing chancroid from syphilitic chancres. The ulcers are shallow with undermined edges and angry red base; they spread rapidly and fresh areas of ulceration develop rapidly by contact infection. The surrounding tissues are not usually toughened, though they may be œdematous. The ulcer is generally more flexible than the primary chancre of syphilis. The inguinal lymph glands are enlarged and tender; there is often redness and induration of the dorsal lymph vessels of the penis. The ulcers increase rapidly in size and may penetrate deeply and a large area of the penis may be destroyed, exposing the urethra. If the patient has a long and tight prepuce the ulcer may become gangrenous (the condition of phagedæna).

Even when the clinical features appear sufficiently distinctive it is advisable to examine the exudate from the sore for *Treponema pallidum*, Ducrey's bacillus and Donovan bodies (granuloma venereum) and the blood should be tested for Wassermann reaction.

Diagnosis. The specific test for chancroid is **Ito-Reensterna's test**. This consists in the intradermal injection of a vaccine of *H. ducreyi* (e.g., Dmelcos vaccine). An injection of 0.2 ml. is made in the forearm and a control injection of 0.2 ml. of sterile saline is made about 6 inches away. A positive reaction consists in the production within 48 hours of an area of redness and swelling about 1 inch in diameter at the site of the vaccine injection. A positive reaction does not necessarily indicate active infection—it is given by all patients who have at some time suffered from the infection. A more direct method of diagnosis is to inoculate a few drops of pus from a bubo into the chorio-allantoic membrane of an 11-day old chick embryo; the organisms are easily demonstrable in the amniotic and allantoic fluids at the end of 48 hours.—K. Anderson and J. S. Snow, *Amer. J. Path.*, 1940, 16, 269.

The identification of the Ducrey bacillus in smears stained by the Unna-Pappenheim method (methyl green pyronine) is the most important single diagnostic criterion of chancroid infection. The intradermal reaction to Ducrey's vaccine was found to be specific and positive in about 95% of cases. Ducrey's vaccine test is most important when results are negative to exclude active chancroid disease. 0.1 ml. of Ducrey's vaccine is injected intradermally—at the end of 48 hours a papule 10 mm. in diameter surrounded by a zone of erythema 5 mm. wide denotes a positive reaction. The test is positive for the first time 8 to 15 days after the appearance of the local lesion. A positive reaction means that the patient has, or has had, a chancroid lesion. There is no reversal of the reaction after successful therapy.—B. A. Kornblith, A. Jordy and L. Chargin, *J. Amer. med. Ass.*, 1/1941, 2150.

Treatment. Specific treatment consists of injections of a vaccine of Ducrey's bacillus and is usually rapidly effective. Intravenous injections are given at intervals of three days and the dose steadily increased. Injections are followed by a pronounced general reaction with pyrexia of about 103°F. If Dmelcos vaccine is not available many cases respond to T.A.B. intravenously in doses of 150 million. For local application eusol baths and soaks repeated every four hours are effective.—Robert Lees, *Practitioner*, ii/1936, 183.

Better results are stated to be obtained with sulphanilamide (see Vol. I, p. 952).

Cholera. The *Vibrio cholerae* or comma bacillus when freshly isolated has the appearance of a short curved rod looking like a comma, but after long subculture the organisms lose their curved shape and are like small granules which stain poorly. They are from 1 to 4 μ in length and about 0.2 to 0.4 μ in breadth, arranged singly, in S-shaped or semicircular pairs, or in short chains; in fluid media spirals are often formed. Freely motile, one terminal flagellum. Gram-negative; non-sporing; non-acid-fast.

Cultivation. The vibrios grow best in presence of abundant oxygen. One of their characteristic features is rapid growth in peptone water (1% peptone with 0.5% of NaCl); a delicate membrane forms on the surface in 6 to 9 hours. Optimum pH, 8.0 to 9.0 (limits for growth 6.4 to 9.6). The optimum temperature is 37° (limits 16° to 42°). They are killed by exposure to 55° for 1 hour, but will survive a temperature of -10° for several hours. They do not rapidly multiply in ordinary sewage, being outgrown by putrefactive bacteria; they are rapidly killed by complete drying and hence are not disseminated in dust.

Hæmolysin Formation. So many factors have been shown to influence hæmolysin formation that it is unwise to place too much reliance on this property as a means of distinguishing *Vibrio cholerae* from non-pathogenic vibrios.

In general, it may be said that the cholera vibrio does not produce a hæmolysin but the El Tor and many non-cholera vibrios can do so. Hæmolytic activity is commonly tested for by growing the organism in broth at 37° for 3 days and then adding 1 ml. of the culture to 1 ml. of a 5% suspension of washed sheep or goat red blood-cells; the mixture is incubated for 2 hours at 37° and the results are read after the tubes have stood in the cold overnight.

Antigenic Structure. The cholera vibrio produces a heat-labile H antigen and a heat-stable O antigen. The H antigen is common to the cholera vibrio and a group of cholera-like vibrios, but the O antigens are more specific and are used as a basis of classification. (For details of the sub-divisions of the heat-stable O antigens see A. D. Gardner and K. V. Vankatraman, *J. Hyg. Camb.*, 1935, 2, 262. These authors suggest that the bacteriological proof of "cholera" or a cholera carrier should rest on the isolation of a non-hæmolytic vibrio with the specific O antigen of Sub-group 1.)

Fermentation Reactions. *V. cholerae* produces acid without gas from glucose, levulose, galactose, saccharose, mannose, mannitol, maltose, starch and dextrin, and different strains vary in their action on lactose

Dieudonné's Medium for facilitating isolation of *Vibrio cholerae* from faeces:—N/1 KOH solution is added to an equal quantity of defibrinated ox blood and heated at 100° for 1 hour. Thirty parts of this mixture are added to 70 parts of nutrient agar made neutral to litmus and the mixture kept for a day or two. When ready for use the medium should have a pH of 9.0 to 9.6—at a lower pH coliform and other organisms grow and the medium is not selective; at a higher pH the growth of the cholera vibrio is inhibited.

Transmission. Under war conditions the cholera "carrier" is undoubtedly the most important factor in spreading infection, though the part played by water, food, fomites, flies and faulty conservancy methods must also be kept in mind. Vibrios have been found in the faeces of flies for 24 to 36 hours after ingestion of infected material by the insects. Whereas vibrios often disappear from the faeces of patients in three or four days, the healthy cholera carrier may go on passing vibrios for two months or longer, though usually the carrier only excretes vibrios for a week or ten days. In India cholera vibrios have been known to live as long as 17 days in stools kept in the dark and with evaporation prevented. The average time of survival is shorter in hot weather, from one to two days in June, compared with seven days in February.—*W.O. Memo. med. Dis.*, 1941.

Prophylaxis. Anti-cholera inoculations not only give a considerable degree of protection, but lessen the risk of a fatal issue. The dose should be at least 12,000 million bacilli given in two injections at 7 or 10 days' interval, and repeated after the lapse of four months. When possible, all indigestible diet should be avoided and special care is needed as regards fruit, raw vegetables and meat jellies; lettuce, celery, melons and cucumbers are especially dangerous.

Treatment. Absolute rest in bed is essential; no food while the disease is active; the surface of the body must be kept warm and intravenous saline given to relieve the cramps; fluid should be given in sips. For the most part drugs are of little use, but the Indian practice of treating the premonitory diarrhoea by giving half-an-ounce of castor oil with a teaspoonful of brandy is probably wise. Kaolin is of value and Tomb's essential oil mixture (see Vol. I, p. 1156) gives encouraging results. The standard treatment for cholera, however, is that of Rogers, which is based on the fact that not only is the water content of the blood reduced by an amount varying from one to two-thirds, but there is also a loss of saline constituents. (For a full description of Rogers' treatment see *W.O. Memo. med. Dis.*, 1941).

Colibacillary Infections. The *Bacillus coli* group includes a variety of types. *B. coli communis* is a normal inhabitant of the intestines, but becomes virulent in certain conditions. It becomes much more virulent in the presence of intestinal infections such as typhoid, amœbic dysentery, bacillary dysentery, etc. The *Bacillus coli* is present in an infant a few hours after birth.

The typical characters of *B. coli communis* are as follows:—Gram-negative bacillus, 2–4 μ long and 0.5 μ broad, producing acid and gas in glucose and lactose broths, acid and clot in milk, indole in peptone water and fluorescence in neutral red.

Cultivation. Pus, or centrifuged deposit from urine, should be inoculated on to MacConkey's bile-salt-lactose neutral-red agar; bile salt inhibits growth of organisms except the coli-typhoid group. Colonies of *B. coli* on MacConkey's medium have a rose-pink colour. To obtain satisfactory growth of other organisms in a mixed infection make cultures on blood agar as well.

Voges and Proskauer's Reaction. Glucose-peptone solution inoculated with culture, incubated 2–3 days; 1 ml. of 10% KOH added and allowed to stand several hours at room temperature. Positive reaction is red fluorescence, due to formation of acetyl-methyl carbinol which in the presence of oxygen and alkali is oxidised to diacetyl; colour attributed to interaction of diacetyl and some component of peptone. This reaction is not given by *B. coli communis*, *B. coli communitor*, *Pneumobacillus* type, or *B. coli anarogenes*; a positive reaction is given by *B. lactis arogenes* and *B. cloacæ* type.

O'Meara's modification is to add to the culture a small quantity of creatin and 5 ml. of 40% NaOH. A pink colour without fluorescence is developed in a few minutes.

Methyl-Red Reaction. Culture in 0.5% peptone, 0.5% dextrose, 0.5% dipotassium hydrogen phosphate and 3 drops neutral red solution (neutral red 0.1 g., alcohol 300 ml., distilled water to 500 ml.) for 3 days. Red colour = "methyl red positive," yellow colour = "methyl red negative." Positive are *B. coli communis*, *B. coli communior*, *Pneumobacillus* type, *B. coli anaerogenes*; negative are *B. lactis aerogenes* and *B. cloacæ* type.

Koser's Medium. 1.5 g. sodium ammonium hydrogen phosphate, 1 g. potassium dihydrogen phosphate, 0.2 g. magnesium sulphate and 2 g. sodium citrate in 1 litre distilled water. *B. coli communis*, *B. coli communior* and *B. coli anaerogenes* cannot utilise salts of organic acids as a source of carbon; hence these do not grow in this medium. Growth (visible turbidity in 10 days) does occur with *B. lactis aerogenes*, *Pneumobacillus* type, and *B. cloacæ* type which can utilise the citrate.

The common types of lactose fermenters from the human and animal intestines have no effect on adonitol and inositol, fail to give the Voges and Proskauer reaction, yield a positive methyl red reaction and fail to grow in Koser's synthetic medium.—Muir and Ritchie.

For further characteristics see *B. typhosus* and Bacteriological Examination of Water, this volume, p. 858.

Cysticercosis. This is the term given to infestation of man by *Cysticercus cellulosæ*, the cystic stage of the tape worm, *Tænia solium*. The adult worm is a parasite of man alone and normally the larval stage is passed in swine, the infested flesh being known as "measly pork." The tapeworm is a rare parasite in England but is common in Asia and many parts of Europe, especially Germany. Primary cases of cysticercosis are rare in this country, but it is now becoming recognised as a common cause of epilepsy in British soldiers returning from service in the East, especially India.

Infestation in man occurs from ingestion of the eggs in uncooked pork or ham, contaminated uncooked vegetables, etc. Within a month of ingestion of the ova the parasite begins to develop in favourable sites, usually the voluntary muscles and the brain, and within 4 months the embryo is fully formed within its cyst, which is about $\frac{3}{8}$ inch long and $\frac{1}{4}$ inch broad. While alive the cyst appears to cause little reaction beyond the development of a fibrous capsule in the adjacent tissues, but on its death, which occurs after an interval of 3 to 6 years or longer, it acts as an irritant foreign body, its disintegration being followed by the liberation of toxic products and swelling of the cyst capsule by fluid.

Normally, three clinical stages may be recognised: (1) *The incubation period.* This is difficult to establish though it should be noted that individuals rarely show signs of the established disease within two years of proceeding to an endemic area. (2) *The period of premonitory symptoms.* These occur during the active life of the parasite and include headache, irregular fever and myalgic pains, though in many cases there is complete absence of such premonitory symptoms. (3) *The established disease.* The most objective symptom of this stage is the finding of palpable subcuticular cysts which are usually about the size of a small pea or bean which may be found in any part of the body and are usually symptomless and which may collapse and disappear within a few days. Epileptic attacks constitute the most important symptom of brain infestation and usually develop only when the cysts have been present for several years. The tendency of the disease is steady retrogression to a fatal termination; other cases progress steadily to a certain stage of chronic invalidism; and a few cases make an apparently complete recovery after a long series of fits. In certain fulminating cases, where there is overwhelming infestation of the brain, death may result within a week from the onset of symptoms, the clinical picture resembling that of an acute encephalitis.—MacArthur, per *W.O. Memo med. Dis.*, 1941.

The following are references to cases of cysticercosis occurring in this country (mostly in soldiers returning from India), recently reported in the literature; W. Blyth, *Brit. med. J.*, i/1941, 401; C. E. Ewing, *ibid.*, ii/1941, 263; T. V. Pearce, *ibid.*, 357; L. J. Segal, *ibid.*, 693; W. E. Dickson and J. D. Willis, *Lancet*, ii/1941, 415.

Diagnosis. Even when nothing can be done for the patient it is important to establish the diagnosis since, in the case of a soldier, it places the illness in a class attributable to military service, and if epileptic symptoms are present it removes the patient's fears of a hereditary taint in his children. The positive diagnosis depends on one or both of two objective findings, namely, the presence of palpable cysts and the calcification of cysts containing dead parasites. To demonstrate the parasite a cyst is excised and examined microscopically; the hooklets on the scolex are diagnostic and characteristic. The radiological examination for calcified cysts is very important, the following regions being radiographed: skull (lateral view), root of neck, upper arms, forearms, thighs, legs. The importance of radiographing the body tissues lies in the fact that cysts in the central nervous system contain so small a quantity of calcium that radiograms frequently fail to reveal them.

The X-ray diagnosis of cysticercosis.—J. F. Brailsford, *Med. Annu.*, 1942, 350; *ibid.*, *Lancet*, i/1942, 127.

Treatment; Treatment is only symptomatic. The fits may be controlled by phenobarbitone and bromides.

Dengue. An acute infectious fever, endemic in certain tropical and sub-tropical countries, being most common in the coastal regions. It is due to a filterable virus transmitted by mosquitoes, *Aedes aegypti* being the principal vector, other probable carriers being *Aedes albopictus* and *Armigeres obturbans*. The patient is infective for the mosquito for the first three days of the disease but the mosquito does not become infective for human beings until the eleventh day after biting; once infected it apparently remains so for the rest of its life. The incubation period is from two to nine days.

The disease is abrupt in onset, with a rapid rise of temperature, which may reach 106°–107°F., and the appearance of a transient rash. There is intense generalised pain, especially in muscles and joints, which at times may be so severe that the patient cannot lie in bed (hence the term "breakbone fever"). There is severe headache and, owing to the ocular muscles being affected, every movement of the eyes causes pain. Insomnia and depression are present and giddiness is common. After three or four days the temperature drops and there is a remission lasting from one to four days during which the patient feels better. This is followed by a relapse, lasting perhaps for a couple of days, and accompanied by the appearance of the terminal rash, which resembles that of measles.

Dengue must be differentiated from influenza (absence of rash), yellow fever (presence of albuminuria) and sandfly fever. It is almost indistinguishable from the last-mentioned and it is thought by some workers that the two diseases are identical.

The differential diagnosis of dengue and influenza.—E. P. Thurston, per *J. trop. Med. Hyg.*, 1933, 344.

Recent epidemics of dengue.—*Brit. med. J.*, ii/1928, 806.

No relationship exists between the virus of dengue and that of yellow fever.—W. B. Sharpe and E. Hollar, *Amer. J. trop. Med.*, 1935, 15, 247.

Treatment. In a recent outbreak in Syria a combination of adrenaline and calcium chloride seemed to influence the whole course and clinical picture of the attack. An injection of 0.5 ml. of a 1 : 1000 solution of adrenaline and 60 gr. of calcium chloride were given daily for five days.—*W.O. Memo. Med. Dis.*, 1941.

Diphtheria. As seen in young cultures, *Corynebacterium diphtheriae* occurs in the form of straight, or, more frequently, slightly curved, rods which measure usually about 3μ to 4μ in length and about 0.5μ in thickness, being rounded or tapered at their ends and staining unequally, the staining occasionally giving a sort of barred marking. They contain granules which produce a beaded appearance and which with certain dyes give a metachromatic action, e.g., staining a purplish tint with polychrome methylene blue. They are stained a deep, almost black, colour with Neisser's and other similar stains. The ends of the bacilli may become swollen, especially in the longer forms; later these may form club-like structures which stain deeply, whilst the protoplasm becomes broken up into globules. Other bacilli may become thicker and segmented, and various stages of disintegration are seen. A characteristic feature in a film is the arrangement of the bacilli, which lie at various angles to one another, giving an appearance similar to Chinese letters or cuneiform characters. Size and general appearance vary with different strains of organisms, with different media, and with the duration of the growth. Sometimes quite sharp types are met with, and rarely, in tryptic digest broth, the culture may consist wholly of coccoid forms, arranged in clumps, diplococcal forms, and chains.—Muir and Ritchie.

Anderson, Happold, McLeod and Thomson (*J. Path. Bact.*, 1931, 667), describe two forms of diphtheria bacilli, *gravis* and *mitis*, the former associated with the severest cases with a high death rate, and the latter with milder attacks and a lower death rate; intermediate forms also occur.

Parish, Whatley and O'Brien do not agree that *gravis* strains are solely, or even mainly associated with severe forms, as they found *mitis* strains at least as virulent, and under laboratory conditions *mitis* strains have produced much better toxins than *gravis*. They suggest instead of *gravis* a non-committal title such as "starch-fermenting type."—*Brit. med. J.*, ii/1932, 915; see also *ibid.*, i/1934, 299.

The cellular morphology of the three types found to be very characteristic, particularly if grown on Douglas's tellurite medium. The "*gravis*" type on this medium is very short, the staining is comparatively uniform and short snowshoe-shaped forms are very characteristic. The "*mitis*" form appears as a slender fusiform rod usually with few, if any, barred forms and with many forms showing metachromatic granules. The "*intermediate*" form is characteristically barred. With practice, cultures can be typed on cellular morphology alone.—C. A. Perry, O. R. Whitley and E. Petran, *Amer. J. Hyg.*, 1936, 23, 580.

From 2160 swabs examined in Cologne 332 strains of *C. diphtheriae* were isolated, and of these 137 were "*gravis*," 31 "*mitis*," and 164 "*intermediate*." In general there was a relationship between type and clinical severity but the relationship was by no means absolute. The "*gravis*" type reaches its maximum growth in 24 hours, and "*mitis*" in 48 hours or more, and the "*intermediate*" in 10 to 24 hours.—K. L. Pesch, *Klin. Wschr.*, 1936, 15, 1202.

In a series of over 6000 cases of diphtheria gathered from many parts of Gt. Britain and Germany during the past five years 95% of the strains of *C. diphtheriae* isolated have fallen into the "*gravis*," "*mitis*," and "*intermediate*" types, described in 1931. The case death rate of "*intermediate*" infections approaches that of "*gravis*" infections and "*intermediate*" strains are at least the equal of "*gravis*" strains in tending to produce haemorrhagic symptoms. The "*mitis*" strains are most commonly associated with laryngeal involvement but apart from this are rarely the cause of death. The discrepancy between the clinical

virulence of the "gravis" strains and their poor powers of producing toxin *in vitro* has so far not yet received an adequate explanation.—K. E. Cooper *et al.*, *Proc. R. Soc. Med.*, 1936, 29, 1029.

Diphtheroids. The diphtheria bacillus is a member of a group of closely related organisms which are widely distributed over the skin and mucous membranes of man and animals. This group corresponds to the genus *Corynebacterium* and has the following characters:—

Gram-positive rod-like forms, usually arranged in a palisade; not acid-fast; often club-shaped swellings at the poles, generally with irregular stained granules; non-motile; no endospore formation; aerobic but often capable of anaerobic growth, not forming gas in carbohydrate media; may or may not produce acidity or liquefy gelatin. Members of the genus *Corynebacterium* other than diphtheria bacillus are termed "diphtheroids" and are widely distributed over the skin, mouth, ear, nose, genitalia, etc. They are met with in health and in many pathological conditions. Their differentiation from *C. diphtheria* is often difficult; some are practically identical with it in morphological and cultural characters and give the typical reaction with Neisser's stain.

Hofmann's bacillus (*Corynebacterium hofmanni*) a non-pathogenic common inhabitant of the throat. Smaller than *C. diphtheria*. Rarely shows beaded appearance; does not give Neisser's staining reaction.

Xerosis bacillus (*Corynebacterium Xerosis*) occurs in xerosis of the conjunctiva, also in nose, throat and ear; morphologically similar to *C. diphtheria*, but grows more slowly on serum (primary cultures from the nose first appear in 36 hours); it is non-virulent to animals; differs from *C. diphtheria* in producing acid from sucrose.

Directions for Collecting Specimens. If a sterile swab is not at hand, a small piece of absorbent cotton wool should be steamed, allowed to cool and rubbed over the membrane on the fauces of the patient and removed in a test-tube or bottle which has been similarly sterilised. If possible, a small portion of the membrane should be detached in addition. The organism may persist for many months in nasal and aural discharges also in dry condition, an important point to recollect in disinfection of bed linen. Moist heat destroys the organism rapidly, e.g., a temperature of 60°. Is also very sensitive to treatment by antiseptics.

Staining. Films are prepared from the swab. Stain by Gram's method (gram-positive) also by Pugh's or Neisser's stains to show metachromatic granules. Dry and mount in xylol balsam.

Neisser's method (modified) of staining the organism:—

- A. Methylene blue, 1 g.
Dehydrated alcohol, 50 ml.
Glacial acetic acid, 50 ml.
Distilled water, 1000 ml.
- B. Crystal violet, 1 g.
Dehydrated alcohol, 10 ml.
Distilled water, 300 ml.

Use a Loeffler's serum culture of 18-24 hours' growth. Stain film for a few seconds in a mixture of solutions A and B, two parts of the former to one of the latter.

Wash for a few seconds in soft or distilled water (this stage may be omitted with advantage). Stain in chrysoidin solution 1:300 for a few seconds (the chrysoidin should be dissolved in warm water and the solution filtered). Wash quickly in water, blot, and dry. The substance of the bacilli is brownish yellow, the granules are almost black.

The results of using Neisser's stain are to be interpreted with caution. Darkly staining granules are not peculiar to the diphtheria bacillus. A not uncommon organism with such a character occurring in the throat is a streptobacillus with square ends; with Neisser's stain it gives an appearance resembling the diphtheria bacillus, but has no resemblance to it in a methylene blue preparation. Some hard waters interfere with the reaction; distilled water ought always to be used for washing the preparations.—Muir and Ritchie.

Pugh's (syn. Ponder's) Toluidine Blue Stain. Toluidine blue 0.02 g., glacial acetic acid 1 ml., dehydrated alcohol 2 ml., water to 100 ml. A loopful of the stain is dabbed on the dried smear and examined as hanging drop with 1/12th in. oil immersion lens. Used for direct examination from the swab, the appearance is characteristic. *C. diphtheriae* appears pale blue with bright and often deeply stained red granules along its entire length, some yeasts and sarcinae also show the metachromatic markings. Hofmann's bacillus stains dark blue with a light band. Diphtheroid bacilli cannot be mistaken or confused with *C. diphtheriae* by the method. It would be well to make the film, if possible, direct from the throat. A negative result is not to be considered of much value. Vincent's angina fusiform bacilli also stain dark blue. The method is claimed to be simple and rapid.—Constant Ponder, *Lancet*, ii/1922, 23.

It can be stated with confidence that in the majority of cases of acute diphtheria from which a satisfactory swab has been taken the disease can be diagnosed on examination of the direct smear. Of 76,000 "acute" swabs so examined during 23 years the diphtheria bacillus has been detected in 85%. It should be remembered, however, that in adults diphtheroid organisms are sometimes seen which resemble the diphtheria bacillus from a child's throat, and it is not advisable to report on direct smears taken from patients over the age of 18. By this method the regrettable "waiting for the result of cultivation" would in many cases be avoided.—C. Ponder, *Brit. med. J.*, ii/1934, 373.

Roux's Stain. Dahlia or gentian violet 0.5 g., methyl green 1.5 g., distilled water 200 g.

Sections of Membrane. Stain for the diphtheria bacillus by the Eosin-Gram method:—

1. Stain 4 or 5 minutes with eosin solution.
2. Wash well in water.
3. Pass through a little alcohol.
4. Stain with aniline-gentian-violet, 10 minutes.
5. Cover with Gram's iodine solution, 3 minutes.
6. Decolorise with aniline oil.
7. Clear with xylol and mount in xylol balsam.

Cultivation. Cultivate on Loeffler's blood-serum (fine cream-coloured growth in 12 to 16 hours) and stain the film from this with methylene blue, Neisser's or Gram's method. Cultivations should in all cases be made on blood serum or glycerine agar before the result of diagnosis can be positive. Further characteristics: no spores, non-motile. Form differs with culture medium.

Glucose Litmus Broth cultures of true diphtheria bacilli show marked acidity in 24 hours, while those of the pseudo-forms are stated not to evince this alteration of reaction. This method is useful for confirmation where no licence for inoculation of animals is held.

Serum-water gives good result: Coagulate blood serum in an equal quantity of water, filter, add to one-half 1% dextrose, and to the other 1% sucrose. Add neutral red as indicator. After 24 hours a marked acidity is produced in the glucose tube by *C. diphtheriae*, in both the glucose and the sucrose tubes by *B. xerosis*, and no change is produced in either tube by Hofmann's bacillus.

Horgan and Marshall's Medium. To 15 ml. of ordinary 2% agar medium at pH 7.6 heated to 50°, add 3 ml. of mixture of equal parts of ox or horse blood and of 2% potassium tellurite solution in distilled water. The blood must have been defibrinated or rendered incoagulable by 1% sodium citrate. The blood-tellurite mixture is poured into plates and is most effective when at least 3 days old. It keeps in the ice-chest without aseptic precautions for 3 weeks. Parish recommends the addition of 0.5% glucose to the digest broth used for the preparation of the agar. A 1.5% solution of potassium tellurite is often preferable, being less inhibitory.—Muir and Ritchie.

McLeod's Medium (The Leeds Medium) for isolation and identification of *mitis* and *gravis* types. Extract 750–1000 g. of minced meat with 1 litre of tap water at 48° for 1 hour. Express fluid through lint, keep in ice-chest overnight and filter through paper. To 1 litre of filtrate add 20 g. of peptone and 5 g. of NaCl, warm to 45° to dissolve. Heat 50 ml. of this solution to 80°–90° for fifteen minutes and filter through paper. Take 10 ml., and determine volume of N/10 NaOH required to make pH 7.6. On basis of this determination add sufficient alkali to the bulk of the medium to bring it to pH 7.6. Filter through Seitz K "clarifying" disc, then through Chamberland candle previously sterilised by autoclaving. For use mix equal parts of this and a melted 5% agar solution in water; add 7 to 10% of fresh defibrinated rabbit blood and 0.04% potassium tellurite. Heat at 75° for 10–15 minutes and pour into plates. (For details of preparation see J. S. Anderson *et al.*, *J. Path. Bact.*, 1931, 34, 667.)

Glass's Medium contains fresh blood and is claimed for that reason to be superior to heated blood media because of the inhibitory effect of the hæmatin contained in the latter on many *mitis* strains.—V. Glass, *J. Path. Bact.*, 1937, 44, 235; 1939, 48, 507.

Smith's Medium. Add 5 ml. of sheep's serum (sterilised at 57°) to 100 ml. of peptone-water-agar (neutral to litmus) melted at 50°; add 0.9 ml. of 1% telluric acid in distilled water. The *C. diphtherie* forms large white colonies after 24 hours' incubation. The growth of many other bacteria is inhibited.

Neill's Blood-Tellurite-Agar Medium. The directions for preparing this medium are given by the author as follows:—

1. *Broth:* Lemco 20 g., "Difco" proteose peptone 20 g., sodium chloride 10 g., distilled water 1000 ml. Dissolve by steaming. Add N/1 NaOH till alkaline to phenolphthalein. Heat at 90° for 30 minutes to precipitate phosphates; filter, adjust pH to 7.6 with N/1 HCl. Bottle in quantities of 100 ml. in 8 oz. screw-top "medical flat" bottles. Stopper tightly; autoclave for 15 minutes at 15 lb. pressure.

2. *Agar:* "Difco" bacto-agar 30 g., distilled water 1000 ml. Dissolve by boiling; bottle in quantities of 100 ml. in 12-oz. "medical flats." Autoclave 20 minutes at 20 lbs. pressure.

3. *Laked Blood Mixture:* A bottle containing 10 g. of sodium citrate in 10 ml. of water is sterilised in an autoclave, stoppered and taken to the slaughter-house; when an ox is being stuck a litre of blood is collected into the bottle through a sterile funnel and vigorously shaken; 1.25 ml. of pure formalin and 30 ml. of methyl ether are added and the bottle is tightly corked.

4. *Potassium Tellurite (1%):* To prepare the medium a bottle containing 100 ml. of the broth is heated in a water-bath at 55°; 10 ml. of the laked blood mixture and 4 ml. of the potassium tellurite 1% solution are added and the whole well mixed. 100 ml. of the agar is melted by immersing the bottle in boiling water and the melted agar cooled to 55°. The broth-blood-tellurite mixture is now added to the 100 ml. of agar and mixed by inverting the bottle gently several times. The temperature of the mixture is now raised in the water-bath to 75° and maintained at that temperature for 15 minutes when the medium is ready for pouring into plates. *Note:* The broth should be heated above 100° on one occasion only. The blood and tellurite should be added to the broth and mixed before the latter is added to the agar.—G. A. W. Neill, *J. Hyg.*, 1937, 37, 552.

Hoyle's Modification of Neill's Medium. Neill's medium was not satisfactory in Cardiff where the types of *mitis* strain present either failed to grow or were much inhibited, and *gravis* strains were rare. To overcome the inhibitory effect of Neill's medium unheated blood agar is used, which gives rapid growth for all types of *C. diphtheria*, with good selectivity. Type selectivity is less satisfactory than McLeod's or Neill's media, but is sufficiently good for experienced workers. The medium is prepared as follows:—

1. *Broth:* Lab. Lemco 10 g., peptone 10 g., sodium chloride 5 g., agar 20 g., water 1 litre. Adjust to pH 7.8 and autoclave. Bottle in quantities of 200 ml. in screw-topped bottles.

2. *Sterile horse blood*, laked by freezing and thawing several times. Preferably stored in frozen state.

3. *Potassium tellurite* 0.7 g., water 20 ml., keep in tightly corked container in the dark.

Melt 200 ml. of (1), cool to 50°; add 10 ml. of (2) and 2 ml. of (3); pour into plates.

Colonies of *C. diphtheria* have a grey colour; good growth occurs after 18 hours incubation.—L. Hoyle, *Lancet*, i/1941, 175.

Instead of laking the blood by repeated freezing and thawing, saponin in a dilution of 1:200 may be used. 0.5 ml. of 10% sterilised solution of white saponin in distilled water is used for each 10 ml. of blood, previously heated in the incubator for 15 minutes. The blood and saponin solution are carefully mixed by inverting the bottle gently, avoiding the formation of bubbles, and reheated in incubator for a further 15 minutes.—Mackie and McCartney.

Bacteriologists who continue to depend entirely upon Loeffler's medium are doing second-rate bacteriological work so far as diphtheria is concerned. The best results are obtained by duplicate examinations, using Loeffler's medium and one of the blood-tellurite-agar media. This will give 10% more positive results and is less likely to miss the most severe cases.—K. E. Cooper *et al.*, *Lancet*, i/1940, 865.

Clauberg's Medium has a great reputation in Germany and has been fully described by Sutherland and Iredale. It is a translucent medium containing unheated laked blood, of highly complex composition and preparation, on which, in virtue of its content of glucose and dyes, diphtheria bacilli develop bright blue colonies. At its best it makes the diagnosis of diphtheria quite a simple matter in so far as all events as the elimination of negatives is concerned (see K. Clauberg, *Zbl. Bakt.*, 1935, 134, 271; 1936, 135, 529; P. L. Sutherland and J. L. G. Iredale, *J. Path. Bact.*, 1937, 46, 325).

It is prepared as follows:—

1. *Glycerinated Blood*: Sterile glycerol 1, sterile defibrinated ox blood 2. Keep six months in refrigerator to ripen.

2. *Dye Solutions*: (a) 2% water blue 6B extra P (Gruebler) in distilled water (methyl blue can be used instead); (b) 2% metachrome yellow II. RD "W" (Gruebler) in distilled water. (Chrome fast yellow G can be used). Shake frequently for 2 days; filter.

3. *Cystine Solution*: 1 g. of anhydrous sodium carbonate and 1 g. of cystine in 10 ml. of boiling distilled water. Make up to 100 ml. with distilled water. Solution should be clear.

4. *Potassium Tellurite Solution*: Dissolve 2.5 g. of finely powdered potassium tellurite (not more than 10 months old) in 250 ml. of sterile distilled water in a sterile flask. Solution should be clear and not used after 6 months.

5. *Placenta Agar*: Three human placentas, minced and added to 4 litres of water, heated to boiling and allowed to simmer 30 minutes and filtered through gauze. To 4 litres of the filtrate add peptone (Witte) 40 g., sodium chloride 12 g., and sodium dihydrogen phosphate B.P. 8 g. Warm to 45° to dissolve. Adjust pH 7.2. Heat in steam; sterilise 15 minutes; filter through paper; adjust pH to 7.2. Add 160 g. agar and heat to dissolve; check pH and store in bottles. When required for use, check pH and melt in steamer; keep at 48° in water-bath.

(A) To 82.5 ml. of fresh sterile ox-blood add 165 ml. of sterile distilled water and stand till lysis is complete; add 11 ml. of glycerinated blood and 18 ml. of potassium tellurite solution. Keep in water-bath at 48°. (B) 0.75 g. sodium acetate, 7.5 g. of glucose, 30 ml. of water blue solution and 10 ml. of metachrome yellow solution and 5 ml. of cystine solution. Keep in water-bath at 48°. Add (B) to (A), mix thoroughly and add 210 ml. of melted placenta agar; mix and pour into plates quickly. After mixing, the heating at 48° should be as short as possible, because potassium tellurite is changed when heated in presence of glucose.

On this medium colonies of *C. diphtheriae* are blue with marginal coloration, colonies of *C. Hofmanni* and *C. xerosis* are yellow, becoming black.

Gundel-Tietz Medium, next to Clauberg's, has had the greatest vogue in Germany. It is prepared as follows:—

To 260 ml. of a 2½ to 3% solution of agar, buffered at pH 7.6, are added 1 ml. of stock cystine solution (prepared by dissolving 0.5 g. of cystine in 5 ml. of a 10% solution of Na₂CO₃ and diluting to 50 ml.) and a mixture of 30 ml. of defibrinated sheep's blood and 12 ml. of a boiled 1% solution of potassium tellurite. The mixture is poured on plates containing very thin layers.—M. Gundel and C. J. Tietz, *Z. Hyg. InfektKr.*, 1934, 116, 439.

For a study of the comparative values of various media for cultivation of *C. diphtheriae*, see K. E. Cooper *et al.*, *Lancet*, i/1940, 865.

Diphtheria Antitoxin. Standard, B.P. The standard is a quantity of dried antitoxin kept in the National Institute for Medical Research.

Unit. The unit is the specific neutralising activity for diphtheria antitoxin contained in such an amount of the standard preparation as the Medical Research Council may from time to time indicate as the quantity exactly equivalent to the unit accepted for international use.

Methods of Assay. The method suggested in B.P. directs those wishing to assay unknown samples of antitoxin to prepare first of all a suitable sample of diphtheria toxin. This is obtained by growing the *C. diphtheriae* on the surface of broth and then killing the organisms by the addition of phenol. Mixtures are then made containing different amounts of this toxin together with 1 unit of antitoxin, and these mixtures are injected into guinea-pigs to see whether they die. The smallest amount of toxin which, when mixed with 1 unit of antitoxin, will cause the death of a guinea-pig in 4 days is said to be the L₁ dose of toxin. Unknown samples of antitoxin are then tested by preparing mixtures containing different amounts of antitoxin with the L₁ dose of toxin

and these mixtures are injected into guinea-pigs. The mixture which causes death in 4 days contains 1 unit of antitoxin.

This method, which has been long established, has been largely replaced in practice by a skin reaction test. The smallest dose of toxin is found which, mixed with 1 unit of antitoxin, causes redness when injected into the skin of a guinea-pig. This dose of toxin is called the L_r dose. Mixtures of the L_r dose with different amounts of unknown antitoxin are then injected to find which mixture just causes a reaction. The amount of antitoxin in this mixture is one unit.

Dysentery. There are two main types of dysentery, amœbic and bacillary.

Amœbic Dysentery results from infection of the colon with *Entamœba histolytica*. In the active vegetative stage the entamœba varies in size from 20 to 30 μ , though it may vary from 18 to 40 μ . A somewhat clear, highly refractile ectoplasm and granular or vacuolated endoplasm can often be distinguished. The nucleus is rounded, about 7 μ in diameter, difficult to distinguish, eccentric, often at the margin of the endoplasm. In fresh material active amœboid movements are seen, and in less active forms pseudopodia are suddenly extruded. Red blood-corpuscles may often be seen in the interior, though this is by no means a constant factor; it is characteristic and of special importance in the recognition of *E. histolytica*. The organism can usually be found in large numbers in the contents of the large intestine in amœbic dysentery. Their real habitat is in the tissues. The lesions of amœbic dysentery are chiefly in the large intestine, but they occur in the lower part of the ileum. Local swellings of the mucous surface are followed by ulceration and gangrenous sloughing. The amœbæ pass through the intestinal wall and on reaching the submucous layer form an abscess. As the stools begin to regain their normal character, the entamœbæ undergo cyst formation. The cysts are spherical, 10–15 μ in diameter, with their walls having double contours. Usually four (sometimes only two) nuclei can be seen in the fresh condition.

To Search Stools and Mucus for *Entamœba histolytica*. The stools must be examined fresh, as soon as possible after being passed, as the amœbæ disappear rapidly. The stool should be passed into a dry vessel and not mixed with urine, water or disinfectant. A piece of mucus may be examined unstained on a warm stage. Some recommend adding one or two drops of 1 in 5000 neutral red in normal saline. Examine with $\frac{1}{2}$ in. objective. The amœbæ take up the neutral red, all other constituents of the fæces, even the leucocytes, remaining uncoloured.

For cysts and precystic forms emulsify a little of the stool (1) with saline and (2) with 1% aqueous solution of iodine in potassium iodide.

Cultivation. Using a medium composed of Locke's solution, egg and serum or albumin, Boeck and Drbohlav cultivated *E. histolytica*. In culture, *E. histolytica* feeds on bacteria and red blood cells whenever the latter are present in the medium. (See *J. Amer. med. Ass.*, ii/1925; 196; *Amer. J. Hyg.*, 1925; *Lancet*, ii/1926, 762; and *Yearb. Pharm.*, 1927, 79; and detection in fæces, *ibid.*, 80, 371.)

Slopes of inspissated horse serum, covered with egg albumen solution and a small amount of sterilised rice starch added, preferred to Boeck and Drbohlav medium.—Dobell and Laidlaw, *Parasitol.*, 1926, 18, 283.

Viability of cysts of *E. histolytica* studied by keeping them in an ice-box and subculturing at intervals; they were viable up to 46 days. Subcultures of Dobell's strains survived under variable conditions of temperature, etc., up to 27 days.—T. and V. Wright, *J. trop. Med. Hyg.*, ii/1932, 337.

Method for counting *E. histolytica* *in vitro* with hæmocytometer.—M. Paulson, *J. trop. Med. Hyg.*, i/1933, 109.

A method of producing encystment in cultures of *E. histolytica*.—W. S. Stone, *Amer. J. trop. Med.*, 1935, 681.

Carriers. It has been shown that *E. histolytica* is not always a tissue parasite; it can occur in the large intestine without tissue penetration. A latent infection does not always mean ulceration of the bowel. In latent carriers the entamæbæ live in the lumen of the gut; they never invade the tissues and they produce only very mild symptoms. It is known that large numbers of apparently healthy people who have never been out of England may harbour *E. histolytica*; indeed, it has been stated that there are nearly two million cyst-passers in this country. It would seem, in fact, that only under certain conditions, of which we are as yet ignorant, does *E. histolytica* manifest itself as a pathogenic agent, and the theory has been proposed that those cysts which may be found in the stools of individuals in countries where amæbiasis is not endemic belong to a closely allied but non-pathogenic race.

The spread and incidence of protozoal infections in the population of this country. As the result of examination of nearly 3000 people, including army recruits, adult civilians, children under 12, and asylum patients (none of whom had been out of England, with the exception of a very small percentage in the last group), *E. histolytica* was found in every section of the population, establishing the wide occurrence of the infection in this country, and showing that it is no longer necessary to presume foreign origin for any home case of amæbic dysentery or for any infection with *E. histolytica*. Indigenous cases of acute amæbic dysentery do occur in England and may be more common than has been supposed—possibly concealed under such names as "hepatitis" or "ulcerative colitis."—A. Malins Smith, *Brit. med. J.*, ii/1924, 897.

Non-pathogenic Amæbæ. Four other species of amæbæ non-pathogenic to man occur in the fæces, namely, *Entamæba coli*, *Endolimax nana*, *Iodamæba* and *Dientamæba fragilis*. These must be differentiated from the tissue-invading and pre-cystic forms of *E. histolytica* in fæcal examinations. The characters of the cysts and of the nuclei of the active forms are the most important distinguishing features.

Comparison of *E. histolytica* with *E. coli*. The following table shows differences from *E. coli*, which is most commonly found in fæces. Actual measurement of size is a great help. Amæbic diarrhœa should never be diagnosed on pre-cystic forms alone.

| | <i>E. histolytica</i> | <i>E. coli</i> |
|---------------------|---|--|
| Active forms .. | Size 20 μ to 30 μ Clear ectoplasm, granular endoplasm Red blood cells included. Sudden explosive movements. Eccentric inconspicuous spherical nucleus with small central chromatic karyosome. | Size 20 μ to 30 μ Ectoplasm and endoplasm both granular. Bacteria, yeasts, vegetable cells included. Movements very slow and no locomotion. Central conspicuous nucleus with an eccentric dot. |
| Pre-cystic forms .. | Size 7 μ to 14 μ , Round. Ectoplasm and endoplasm not differentiated. No inclusions. No movement. Nucleus a beaded ring. May be elongated and undergoing division. | Almost impossible to distinguish from <i>E. histolytica</i> . |
| Cysts | Size 7 μ to 9 μ , or 11 μ to 14 μ . Mature cyst has 2 to 4 nuclei. Elongated or "chromatoid" bodies present. Droplets containing glycogen. | Size 15 μ to 20 μ . Mature cyst has 2 to 8 nuclei. No elongated "chromatoid" bodies. Abundant glycogen. |

Endolimax nana (formerly called *Entamoeba nana*) is a small amoeba measuring when rounded 6μ to 12μ , somewhat resembling a small *E. coli* in its appearance and movements. The nucleus has large karyosome. Protoplasm has vacuoles containing ingested bacteria. Cysts about same size as free forms, with 4 nuclei, glycogen and small granules.

Iodamoeba. $8-12\mu$ in diameter, resembles small specimens of *E. coli*; nucleus vesicular with large central karyosome. Cysts about same size, single nucleus, abundant glycogen.

Dientamoeba fragilis. Rare. $5-11\mu$ in diameter. Usually 2 nuclei but un-nucleated forms occur; no cysts formed.

Bacillary Dysentery. The bacteria of the dysentery group known to be causative agents of dysenteric symptoms are *B. dysenteriae* Shiga, *B. dysenteriae* Schmitz, *B. dysenteriae* Flexner and *B. dysenteriae* Sonne. They occur as short rods $1-2\mu \times 0.5\mu$, destitute of flagella and non-motile; non-spore-forming and non-capsulated; Gram-negative. Grow freely in all usual media, aerobically; facultative anaerobe. Optimum temperature for growth is 37° , and optimum pH $7.0-7.6$. On solid media colonies are greyish and semi-translucent; in liquid cultures there is uniform turbidity and no pellicle formation. Gelatin is not liquefied.

Sonne Dysentery is the commonest of the three types (Shiga, Flexner and Sonne) in this country but runs the mildest and shortest course. It is most common in the summer months, though it may occur at other times, and no age is exempt. The mode of transmission has not yet been determined and thus, as in typhoid fever, patients and those in attendance on them must be regarded as the source of contamination of food, drink, feeding utensils, etc. Outbreaks of Sonne dysentery usually arise in a children's ward from the admission of a case in which the stools are not typical of the disease. Thus very mild cases and convalescent cases constitute the chief danger.

The disease develops with great rapidity, with onset of fever up 102° to 103° , abdominal pain with colic and perhaps tenesmus, and purging; vomiting may be absent, but is common in the early stage of the disease. By the second day the more violent symptoms are subsiding, and by the end of the third day the temperature is falling rapidly, blood is disappearing from the stools, pain is lessening, and the number of stools diminishing. There is a rapid recovery to normal health within about 10 days.

The bacteriological diagnosis is of the utmost importance and a reliable report can be obtained in 18 hours. A piece of mucus is picked out of the stool, gently washed free of faecal matter in normal saline and spread over a small area of a warm dry plate of lactose-litmus-bile-salt-agar or similar medium. Any surplus material is removed from the surface, and from this area, by means of a spreader, the rest of the plate is planted out by quartering. Eighteen hours later a suspicious colony is removed and emulsified in water on a slide. A loopful of Oxford specific agglutinating serum for the Sonne bacillus is added and if the test is positive macroscopic clumping is seen within a minute. The use of anti-dysenteric serum is neither necessary nor useful in Sonne dysentery.—R. Miller, *Brit. med. J.*, i/1938, 64.

Report of a ward epidemic of Sonne dysentery in which 15 out of 27 patients in a children's ward contracted the infection and 5 had relapses.—F. Pygott, *Lancet*, i/1938, 255.

Dysentery due to *B. dysenteriae* Schmitz. The first known outbreaks in Great Britain in which the Schmitz bacillus was identified as the causal agent occurred in North Wales between 1935 and 1938. From 21 out of 29 patients in the 1935 outbreak an organism was isolated having the morphologically cultured characteristics of *B. dysenteriae* Schmitz and in the 1938 outbreak the organism was identified by its serological reactions as well as morphological and cultural characteristics; it was found in 39 of 62 cases examined.—A. C. Evans, *Lancet*, ii/1938, 187.

Dysentery due to *Bacterium Alkalescens*. Evidence was collected from the literature to show that *Bact. alkalescens* is pathogenic for man; 17 cases

are recorded in which *Bact. alkalescens* has been isolated from the faeces; in these it was associated with disease, nearly always involving the intestinal tract. One example is given of case to case infection in a ward showing infectivity of organism. The authors conclude that the organism can produce a mild form of acute dysentery or chronic colitis. The infection is thus primarily intestinal and lesions produced elsewhere are to be regarded as complications such as follow Flexner dysentery. Specific agglutinins of significant titre were produced in many cases. *Bact. alkalescens* shares certain antigens with other dysentery organisms. Symptomless carriers occur relatively more often than after Flexner dysentery. This may explain why complications due to a secondary invasion of the urinary tract or blood stream, for example, are relatively more common.—D. Nabarro and D. G. Edward, *J. Path. Bact.*, 1939, 49, 575.

Experiments to determine the pathogenicity of *Bact. alkalescens* for rabbits, guinea-pigs and mice showed that relatively large amounts of cultures were necessary to produce death or symptoms. Similar effects were produced with filtrates or autolysed broth cultures. It was concluded that although *Bact. alkalescens* appears to be just as toxic for animals as *Bact. dysenteriae* Flexner, the toxicity is due to an exotoxin similar to those that can be extracted from other Gram-negative organisms, both pathogenic and non-pathogenic, and these experiments, therefore, afford no contributory evidence for believing *Bact. alkalescens* to be pathogenic for man.—D. G. Edward, *J. Path. Bact.*, 1940, 51, 245.

Ciliate Dysentery (Balantidiasis) resembles amoebic dysentery, but is caused by *Balantidium coli*, a ciliate found in pigs, apes, baboons, rhesus monkeys and man. Mode of infection not known, but commonest among workers in occupations associated with pigs. *Balantidium coli* is pear-shaped, 50–80 μ \times 30–50 μ ; cilia in longitudinal rows, vegetative forms found in fluid stools, or in material removed by curette from the base of an ulcer; encysted forms occur in solid stools.

Flagellate Diarrhoea. *Giardia intestinalis* (*Giardia lamblia*), *Trichomonas hominis* and *Chilomastix mesnili* are flagellates encountered in the intestine of man. Their rôle in the causation of dysenteric symptoms has not been proved. The free flagellates adapted to a liquid environment are found only in the stools of patients suffering from diarrhoea, but the encysted forms of *Giardia* and *chilomastix* occur in normal stools (*Trichomonas* does not form cysts).

In adults infection of the duodenum and jejunum with *Giardia* may be associated with recurrent diarrhoea; in children the infection is associated with the passage of pale bulky stools containing mucus and undigested food remnants.

In a case of giardiasis in a boy the symptoms and findings resembled those of idiopathic steatorrhoea. The parasite was found in the stools only after a severe saline purgation, after which the steatorrhoea and abdominal pains disappeared and the parasite was not subsequently observed.—J. McGrath and S. J. Boland, *Brit. med. J.*, ii/1942, 4.

An outbreak of enteritis, much of it of a chronic type, affecting a high proportion of both children and adults in a residential nursery for evacuees is described. *Giardia lamblia* was found in 71% of children and adults having loose stools at the time of examination and in only 32% of those having normal stools; and in 82% of those with a history of intermittent or continual loose stools for some months compared with 25% of those without such a history. Cure of the infection was quickly and successfully accomplished by the use of Quinacrine in two 5-day courses.—G. Ormiston *et al.*, *Brit. med. J.*, i/1942, 151.

Classification. The non-mannitol-fermenting group includes *B. dysenteriae* Shiga and *B. dysenteriae* Schmitz; the mannitol-fermenting group includes *B. dysenteriae* Flexner sub-group and a number of related organisms, e.g. *B. alkalescens* sub-group and *B. dysenteriae* Sonne. *B. dysenteriae* Shiga produces a very potent toxin and is associated with the most severe form of bacillary dysentery. Cultures do not ferment lactose, mannitol or dulcitol; in glucose acid is produced but no gas. It differs from *B. dysenteriae* Schmitz in not producing indole in peptone water. *B. dysenteriae* Schmitz is now accepted as a pathogenic cause of dysentery, although it was formerly regarded as only a secondary invader. It resembles *B. dysenteriae* Shiga in its fermentation reactions, but produces indole in peptone water.

The mannitol-fermenting group of *B. dysenteriae* are responsible for the majority of cases of bacillary dysentery, although they are less toxic and produce symptoms of a milder type than those produced by *B. dysenteriae* Shiga.

The Flexner sub-group are non-lactose fermenters, in glucose and mannitol they produce acid and gas but do not ferment dulcitate.

The *B. alkalescens* sub-group are also non-lactose fermenters, but in glucose and mannitol they produce acid without gas; in dulcitate acid production occurs after incubation for periods of 4 to 8 days.

The Sonne sub-group acidify lactose after incubation for 4-8 days or longer, produce acid but no gas in mannitol and glucose, and do not change dulcitate.

(See also J. S. K. Boyd., *Trans. R. Soc. trop. Med. Hyg.*, 1940, 33, 553.)

Serological Classification. Andrews and Inman described five races in the Flexner group, which they designated V, W, Y, X and Z. Each of these has a distinctive type antigen, but they all have a common group-antigen. (The Y race corresponds generally to the Hiss-Y type of Flexner bacillus described by Hiss and Russell.—Muir and Ritchie.)

Diagnosis of acute bacillary dysentery depends upon the isolation of the causative organism from the faeces. The specimen must be obtained as early as possible in the disease and must be either absolutely fresh or preserved with 30% glycerin in saline of pH 8. Microscopical examination of the mucus may often lead to a provisional diagnosis. The bacillus can be isolated by plating mucus on MacConkey's medium or litmus-lactose-taurocholate-agar, or, preferably, Wilson and Blair's medium (*infra*) and identified by biochemical and serological tests.

Cultivation. On litmus-lactose-agar and on the media of MacConkey, Conradi-Drigalski and Endo, the unrestrained growth of *B. coli* leaves little place on the plates for the development of dysentery bacilli, so that their successful isolation is usually possible only in the early acute stages of the disease and when the stools are planted out within a few hours of their collection. To the antiseptic action of such dyes as malachite green, methyl green, victoria green, brilliant green, fuchsin, crystal violet and gentian violet, dysentery bacilli are more sensitive than most strains of *B. coli*.

Wilson and Blair's Medium. A medium on which Flexner's *B. dysenteriae* grows profusely, whereas there is great inhibition of most strains of *B. coli* and *B. lactis aerogenes*, and suppression of typhoid and paratyphoid bacilli, Sonne's bacillus and cholera vibrios, is prepared as follows:—

To 100 ml. of melted nutrient agar (pH 7.4) cooled to 60° are added 0.5 ml. of 1% rosolic acid in absolute alcohol, 1 ml. of 4% aqueous solution of iron alum and 3 ml. of a lactose-tellurite solution. The latter is made by boiling 20 g. of lactose in 100 ml. of distilled water, cooling and dissolving in the cold solution 0.2 g. of potassium tellurite. (Reduction occurs unless the tellurite is dissolved in the cold.) The solution keeps for weeks, the tellurite being an effective preservative against bacteria, but not against moulds; growth of the latter can be prevented by adding 3% ether and keeping the bottle tightly stoppered. Five strains of Flexner's bacillus (V, W, X, Y, Z) all grew well on this medium.—W. J. Wilson and E. M. McV. Blair, *Brit. med. J.*, ii/1941, 501.

These authors also described another medium prepared as follows: 0.5 g. of sodium tauroglycocholate and 0.5 g. of lactose are dissolved in about 10 ml. of boiling water in 100 ml. wide-necked extraction flask; melted nutrient agar cooled to 60° is added to 100 ml., followed by 0.5 ml. of 1% potassium tellurite, 2 ml. of 4% iron citrate and 0.5 ml. of 1% aqueous neutral red solution. On the surface of this medium, colonies of Flexner's bacillus are yellowish and any strains of *B. coli* that are not suppressed are reddish. Stools which show only a few colonies of *Streptococcus faecalis* on ordinary MacConkey's medium provided abundant tiny colonies on this medium. There is no danger of confusing them with colonies of Flexner's bacillus, but their suppression would be an advantage. This can be done by addition of 0.5 ml. of 1% rosolic acid in absolute alcohol.—*ibid.*

Results from the use of Wilson and Blair's tellurite-iron-citrate-neutral-red medium on 20 consecutive stools from which Flexner dysentery bacilli were cultivated confirm the value of this medium. It will sometimes have an advantage of 2 or 3 days over MacConkey's medium in reporting a negative result. This high selectivity must be kept in mind lest other pathogens be missed.—D. B. Bradshaw, *Brit. med. J.*, i/1942, 181.

Colonies of *B. dysenteriae* Flexner were easily recognised and growth in most of the positive cases was profuse. Colonies were pinkish and glistening when viewed against the light, but against a white background they had a greyish tinge. Most of the *B. coli* group were inhibited, but organisms of the proteus and alkaligenes groups and also some enterococci grew fairly well. In most of the acute cases a pure culture of dysentery organisms was obtained. There is sufficient evidence to indicate the value this medium is likely to have in the isolation of suspected cases of dysentery and of carriers.—J. C. S. Thomas and W. A. Hulme, *Lancet*, i/1942, 321.

Leifson's Desoxycholate-Citrate-Agar Medium. Fresh lean minced pork infused for an hour in three times its weight of distilled water (break up the lumps of meat and distribute them evenly). Add 1 ml. of N/1 HCl for each 100 g. of meat and boil the mixture for one minute. Strain, and filter through paper until free from visible fat droplets. Add a volume of N/1 NaOH equal to volume of N/1 HCl added. Again boil for one minute, filter through paper. Add distilled water to make up volume to that of water originally added.

To this infusion add 1% peptone and NaOH *q.s.* to make pH 7.5. Boil 2 or 3 minutes, filter. Add 2% agar and to each 100 ml. of medium add 0.5 ml. of N/1 NaOH. Allow the agar to soak for 15 minutes, then dissolve by boiling or autoclaving. Then add lactose 1%, sodium citrate ($\text{Na}_2\text{C}_6\text{H}_5\text{O}_{11}\text{H}_2\text{O}$) 2.5%, sodium desoxycholate 0.5% and lead chloride (1:300,000) as rapidly as possible. If not required for immediate use, the medium should be stored without further addition. For use, melt at 80°–100° and add 0.2% of ferric ammonium citrate (green). Titrate to pH 7.4, using phenol red indicator and add neutral red (1:50,000). Pour into petri dishes and protect from light and drying.—E. Leifson, *J. Path. Bact.*, 1935, 40, 581.

This medium is useful for the isolation of intestinal pathogens, e.g., *B. typhosum*, *B. paratyphosum*, *B. dysenteriae* Flexner, *B. suptistifer*, and all Gram-positive bacteria. Some strains of paratyphoid bacilli, the Shiga, Sonne, *dispar* and *alkalescens* types of dysentery bacilli are inhibited. *Vibrio cholerae* grows poorly, *B. pyocyaneus* grows well, *B. alkaligenes* usually does not grow; most strains of *B. proteus* grow and most strains of colon bacilli are inhibited to a large extent.—Mackie and Macartney.

Dysentery Carriers. Healthy carriers of *B. dysenteriae* do not occur. Actual carriers are to be found among the incomplete convalescents in whom there is always evidence of an unhealed ulcer.

There is a growing certainty that dysentery due to the bacilli of Sonne, or Flexner, or Shiga is endemic in this country and that much of it escapes notice. Many cases of illness described as gastro-enteritis, gastric influenza and colitis would undoubtedly be more precisely described as cases of bacillary dysentery if proper laboratory examination of the stools was made in the acute stage of the disease. Hospital staffs in children's homes or hospitals should always be on the alert for its appearance and any child brought to hospital suffering from severe diarrhoea should be kept under observation and not allowed into a general ward until bacteriological examination has been made to exclude the presence of dysentery bacilli in the faeces.—*Rep. med. Offr Minist. Hlth, Lond.*, 1936, 39.

Antidysentery Serum (Shiga). *Standard, B.P.* The standard is a quantity of dried serum kept in the National Institute for Medical Research, Hampstead.

Unit. The unit is the specific neutralising activity for the *B. dysenteriae* (Shiga) contained in such an amount of the standard preparation as the Medical Research Council may from time to time indicate as the quantity exactly equivalent to the unit accepted for international use.

Method of Standardisation. A preparation corresponding to a test toxin is first made. A toxin is, however, by definition a poisonous substance liberated in a culture medium by a growing organism, and the preparation made from *B. dysenteriae* Shiga is not always a toxin in this sense. It may be a bacillary emulsion, prepared by growing a smooth strain of a highly toxigenic *B. dysenteriae* on nutrient agar for 48 hours at 37°. The growth is washed off the medium and the suspension is killed by heating to 56° for 15 minutes and then centrifuged to collect the dead bacilli. These are dried *in vacuo* over P_2O_5 , and ground to a powder. The test toxin may also be a broth filtrate prepared by growing the bacillus in alkaline broth at 37° for 2 or 3 weeks. The broth is

sterilised by filtration, and the toxin is precipitated by adding 40 g. of ammonium sulphate to each 100 ml. The precipitate is again dried *in vacuo*. Finally, a purified toxin may be prepared by dialysing the sterilised broth filtrate under pressure against water. The reaction is then adjusted to the isoelectric point to precipitate the toxin which is collected and dried.

When the toxin is prepared the test dose is determined; this is the dose which when mixed with 1 unit of standard antitoxin causes the death of one half of a group of mice injected with it; a suitable toxin has a test dose which is not less than 20 average lethal doses. Unknown samples of antitoxin are then estimated by mixing different volumes with the test dose of the toxin. These mixtures are injected into groups of mice, and the mixture which kills half of a group of 30 mice contains an amount of antitoxin equal to 1 unit.

Epidemic Encephalitis (previously called **Encephalitis lethargica**). The causal organism is a filter-passing virus. The symptoms are sometimes like influenza, for which it is often mistaken. Cases that are rapid in onset, with high fever, headache, delirium and convulsions are usually rapidly fatal. In the cases of insidious onset there is moderate pyrexia, headache, catarrhal symptoms, pain in the trunk and limbs, and giddiness. There may be hiccough and vomiting. Nervous symptoms usually appear after some days and include disturbances of vision, sleeplessness and alteration of the sleep rhythm. The acute stage may be so mild as to escape detection. Late effects make their appearance after months or years. In the chronic stage there may be mental changes, parkinsonism, sleep disorders, paralysis, tics or disorders of vision, especially oculogyric crises.

Parkinsonism, a form of paralysis agitans, is a common sequel even in young children—characterised by rigidity of musculature of body, the face becoming devoid of expression, and the speech affected; as the stiffness increases the patient becomes unable to feed or dress himself and finally may become bedridden. Further sequelæ are neurasthenia, intractable insomnia and disturbances in respiratory mechanism. Moral degeneration often follows the disease.

Incidence. Whereas the number of cases reported in England and Wales was 5000 in 1924, only 1036 cases were reported in 1929, and during the ensuing ten years there was a progressive reduction, the figure for 1938 being 194. As an acute disease it has largely ceased to be a problem, but the late results continue to appear and the mortality statistics do not show the same progressive reduction as the notifications. Thus, the number of deaths in 1929 was 1037 and in 1938, 650.

Treatment. The only drugs still spoken of favourably when used as palliatives in certain phases of the chronic condition are atropine and its derivatives, bulbocapnine, hyoscine (scopolamine), and stramonium.

Filariasis. The term filariasis is used to denote infestation of man by nematodes of the family *Filarioidea*, which are thread-like worms usually found in the lymph-vessels and glands, the connective tissue and the body cavities. Several species are known to infest man; of these the most important are *Wuchereria bancrofti* (*Filaria bancrofti*), *Loa loa* and *Onchocerca volvulus*.

Wuchereria bancrofti. This is the most important of the filarial worms and is especially common in India, South China, the West Indies and the Pacific Islands. The females are about 75 mm. \times 0.25 mm. and the males about 40 mm. \times 0.1 mm. The female gives birth to enormous numbers of larvæ which make their way through the lymphatics to the blood stream,

living in the lungs and thoracic blood vessels by day and appearing in the peripheral blood only at night (nocturnal periodicity). The intermediate hosts are mosquitoes, chiefly *Culex fatigans* and *Aedes variegatus*: these suck the embryos out of the blood at night and the embryos after development in the thoracic muscles of the insect are inoculated into man.

The infection in most cases is symptomless unless there is obstruction to the lymph flow, when it may give rise to a variety of conditions such as high fever, lymphangitis, abscess, varicose groin-glands, varicose axillary glands, orchitis, funiculitis, synovitis, chyluria, hydrocele, elephantiasis and chylous ascites.

Loa Loa. This parasite occurs commonly in West Africa. The adult worms live in the subcutaneous tissue and the embryos have a diurnal periodicity, as distinct from the nocturnal periodicity of *W. bancrofti*; the intermediate hosts are a species of flies (*Chrysops dimidiata*, *C. longicornis* and *C. silacea*) which feed during the daytime and in which a cycle of development occurs, similar to that of *W. bancrofti* in the mosquito. The parasite gives rise to transient subcutaneous swellings, about the size of a hen's egg and known as Calabar swellings and the worm occasionally migrates to the eye, causing local irritation and swelling.

Onchocerca volvulus. This worm, which is found on the West Coast of Africa, and in Guatemala and southern Mexico (where it is known as *O. caecutiens*), inhabits the subcutaneous or connective tissues of man. The intermediate hosts are a species of flies (*Simulium*). The worm gives rise to subcutaneous tumours in various parts of the body and in chronic cases may penetrate into the tissues of the eye eventually causing blindness (hence "blinding filaria").

Diagnosis. This usually depends on demonstrating the larvæ in the blood (in the case of *O. volvulus* in the fluid from an excised nodule); thick films should be made and stained with Giemsa. A complement fixation reaction may be obtained in most cases by using an antigen prepared from *Dirofilaria immitis*, the heart-worm of the dog.

Treatment consists in careful removal of the worm or, in the case of *O. volvulus*, excision of the nodules.

Chopra and Rao have tested a large number of drugs for their influence on filarial infection. Of these, Fouadin gave the most satisfactory results, causing disappearance of the circulatory microfilariae which was, however, only temporary. It succeeded in controlling the inflammation and fever for a comparatively long time, and this result was also obtained with Soamin, but this drug failed to bring about any appreciable diminution in the number of circulating microfilariae.—per *Trop. dis. Bull.*, 1941, 491.

Gas Gangrene. An acute spreading gangrene, characterised by the development of large amounts of gas in the tissues and by a profound toxæmia. It is due to the infection of wounds by spore-bearing anærobes, *Clostridium welchii*, *Cl. septique*, *Cl. œdematiens* and *Cl. histolyticum*. Usually within 48 hours of the injury the wound becomes abnormally painful and is surrounded by much swelling; the pulse-rate rises rapidly and there may also be a rise in temperature. The diagnosis of gas gangrene is based on the severe and progressive toxæmia, rapid pulse, restlessness, euphoria and vomiting, and an increasing swelling and tension in the region of the wound, with evidence of gas in the tissues.

Wounds in which gas gangrene develops are those involving damage to a large mass of muscle, confined in such a way that the products of infection cannot escape. Small wounds only develop gas gangrene if the damaged tissue is muscle. The infection is common in war wounds but less common in civil practice.

The majority of cases of gas gangrene are caused by *Cl. welchii*, either alone or in combination with other anærobes. About 40% of human gas gangrene is caused by *Cl. œdematiens* alone or in combination with other bacteria. *Cl. septique* as a cause of human gas gangrene may be estimated as 10–20% according

to statistics based on sound bacteriological data. All *welchii* and *Septique* strains must be considered as pathogenic, but only two-thirds of *oedematiens*.—per *Bull. War Med.*, 1942, 3, 166.

***Clostridium welchii* (B. *perfringens*).** Anaerobic, non-motile, spore-forming organism, about 4–6 μ long, fairly broad; Gram-positive (in old cultures may be Gram-negative); usually capsulated in smears from tissue fluids or from cultures in serum media, no capsule in cultures from agar or broth media. Liquefies gelatin, ferments glucose, maltose, lactose, sucrose and starch, with production of acid and gas. In litmus milk medium produces characteristic "stormy clot"; only feebly proteolytic. Produces an exotoxin in fluid cultures.

Nagler Reaction for Identification of *Cl. welchii*. The reaction with litmus milk (*supra*) may be falsely negative as some strains of *Cl. welchii* do not form clot or because the amount of culture used to inoculate the milk is too small. The reaction may be positive in the absence of *Cl. welchii*, especially with mixtures of aerobes and anaerobes.

The production by *Cl. welchii* toxin of turbidity and sometimes a fine curdiness in human serum constitutes a possible diagnostic test for *Cl. welchii*, by which a positive specific result may be obtained within 2, or perhaps 3, days of taking the specimen. The reaction has the advantage of being serologically specific, as it is inhibited by *Welchii* antitoxin. The technique of the test is as follows (N. J. Hayward, *Brit. med. J.*, i/1941, 811):—

To one of two sterile 6 mm. (Wassermann) tubes is added 0.3 ml. of a mixture of equal volumes of human serum and Fildes' peptic blood digest broth (see p. 913); to the other tube is added, as a control, 0.3 ml. of a similar mixture containing in addition 10% of concentrated *Welchii* antitoxin. (The test and control-mixture are conveniently made in bulk and kept at 2°.) Each tube is inoculated with either a drop of culture or half a suspected *Cl. welchii* colony and is observed after 16, 40 and 64 hours' anaerobic incubation at 37°. In a positive reaction the serum becomes turbid and in a strongly positive reaction a fine yellowish curd, which tends to float on the surface of the fluid, is produced. On spinning, the material separates into three zones—a compact deposit of bacilli, a more or less compact scum of fat-like material and an intermediate fluid that is either turbid or clear, apparently depending on the packing of the fatty material in the top layer. When only a small amount of toxin is produced, spinning does not cause the formation of a scum, but the fluid above the bacterial deposit is turbid. Reactions are recorded as ++ or + according to whether or not a well-defined top layer is produced. The control tube shows turbidity due to bacteria only and on spinning forms a compact deposit at the bottom of a clear fluid. A single colony supplies ample material for the inoculum.

The authors also used solid media. Fildes' agar with 10–20% human serum gave, after 16 hours anaerobic incubation, colonies with clearly defined zones of opacity 2–4 mm. wide; these were inhibited in control plates containing antitoxin. Positive Nagler plate reactions were often obtained within 24 hours of taking a swab from infected wounds.

For routine rapid identification the test should be applied to any colony suspected of being *Cl. welchii*, for there is a very good chance of obtaining a result in 24 hours. At the same time as the Nagler test is set up, a meat broth culture should be sown and used for a heavy inoculum in a second test if the first test is negative after 24 hours; this will probably yield a result by the second day.

Variants of *Cl. welchii*. The name *Cl. welchii* is now applied to several closely-related variants associated with diseases of man and animals, characterised by general toxic symptoms as well as local lesions in the intestines and elsewhere; these bacterial variants are classified into four types designated types A, B, C and D. Type A is mainly associated with gas gangrene in man; Type B (*Cl. welchii*, var. *agri*) causes lamb dysentery; Type C (*Cl. paludis*) occurs in "streak," an acute enteritis of sheep, and Type D (*Cl. ovinotoxicum*) causes infectious entero-toxæmia of Australian sheep, "pulpy kidney" disease of lambs and probably grass-sickness of horses. These variants differ in the specific character of the toxin present in the filtrates of fluid cultures; each type of organism may form several distinct toxins, and the same toxic constituent may be formed by two or more of the types.

Toxin-Antitoxin Neutralisation Reactions of the Types of Cl. welchii.

| Toxic filtrates from Types | <div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle;">{</div> <div style="display: inline-block; vertical-align: middle; text-align: center;"> A B C D </div> </div> | Antitoxins Against Types | | | |
|-------------------------------|---|--------------------------|---|---|---|
| | | A | B | C | D |
| | | + | + | + | + |
| | | — | + | + | — |
| | | — | + | + | — |
| | | — | + | — | + |

For further details see A. J. Wilsden, *Univ. Camb. Instit. Animal Path.*, 2nd Report, 1931, 53, and 3rd Report, 1932, 46.

Toxins of *Cl. welchii*. *Alpha* toxin is characteristic of Type A, responsible for gas gangrene in man; it has hæmolytic, necrotising and lethal properties; it is also formed by Type B and Type C. *Beta* toxin is necrotising and lethal, but not hæmolytic; it is produced by Types B and C. *Gamma* toxin is lethal to mice and is produced by Type B.—Glenny *et al.*, *J. Path. Bact.*, 1933, 37, 53.

For a discussion of the action of *Cl. welchii* (Type A) toxin on red corpuscles, striated muscle, the circulation, the adrenal glands and tissue oxidations, see G. Payling Wright, *Bull. War Med.*, 1942, 4, 251.

A modified liver and veal digest broth without commercial peptone for the production of a very potent *Cl. perfringens* (Type A) toxin is described. Studies of the lethal hæmolytic and opacity factors contained in the toxin indicate that possibly a more complete perfringens toxin (Type A) is essentially composed of two antigens each of which is characterised by a different type of hæmotoxin.—S. C. Seal, *Indian J. med. Res.*, 1942, 30, 229.

Gas-gangrene Antitoxin (Perfringens). *Standard, B.P.* The standard is a quantity of dried gas-gangrene antitoxin (perfringens) kept in the National Institute for Medical Research.

Unit. The unit is the specific neutralising activity for gas-gangrene (perfringens) toxin contained in such an amount of the standard preparation as the Medical Research Council may from time to time indicate as the quantity exactly equivalent to the unit accepted for international use.

Method of Comparison. The following is an abbreviated account of the method suggested in the B.P. A test toxin is prepared by growing *Cl. welchii* for 16 hours; the medium is sterilised by filtration and ammonium sulphate is added until a precipitate is formed which is collected and dried. The test dose of the test toxin is determined by finding what amounts of the test toxin, mixed with 0.2 unit of standard antitoxin, kills some but not all of a group of mice into which it is injected intravenously. Unknown samples of antitoxin are then assayed by making mixtures of different volumes with the test dose of the test toxin. These mixtures are then injected into mice, and the one which kills some but not all of the mice contains an amount of antitoxin equal to 0.2 unit.

Vibron septique. An anaerobic spore-forming, motile organism, occurring in rods 3 to 10 μ in length and about 1 μ thick, may grow into long segmented filaments in fluid media. Several peritrichous flagella are present; it is Gram-positive, but old cultures may be Gram-negative. Spores are oval, central or subterminal. Liquefies gelatin, but not coagulated serum. Ferments glucose, maltose and lactose; does not ferment sucrose, inulin, glycerol or starch; only feebly proteolytic. Produces an exotoxin in fluid cultures.

Gas-Gangrene Antitoxin (Vibron septique). *Standard, B.P.* The standard is a quantity of dried gas-gangrene antitoxin (vibron septique) kept in the National Institute for Medical Research, Hampstead.

Unit. The unit is the specific neutralising activity for gas-gangrene (vibron septique) toxin contained in such an amount of the standard preparation as the Medical Research Council may from time to time indicate as the quantity exactly equivalent to the unit accepted for international use.

Method of Comparison. The following is an abbreviated account of the method suggested in the B.P. *Add. I.* A test toxin is prepared by growing

Vibrio septique for from one to three days; the medium is sterilised by filtration and ammonium sulphate is added until a precipitate is formed which is collected and dried. The test dose of the test toxin is carefully determined in one of two ways, either by intravenous injection into mice or by intracutaneous injection into guinea-pigs. The test dose is the least amount of toxin which mixed with 1 unit of antitoxin kills some but not all of the mice which receive it; alternatively it is the least amount which mixed with 0.5 unit of antitoxin causes a reaction in the skin of the guinea-pig. When the test dose is determined by one of these methods, unknown samples of antitoxin are estimated by the same method. Mixtures are made so that 0.5 ml. contains in normal saline solution 0.2 ml. of toxin solution and different quantities of the antitoxin being tested. The mixture of the antitoxin being tested which contains 1 unit in 0.5 ml. is that which kills the same, or most nearly the same, number of mice as the mixture which contains 1 unit of antitoxin in 0.5 ml.; alternatively, that mixture which contains 0.5 unit in 0.2 ml. is that which produces the same degree of local reaction in guinea-pigs as the mixture containing in 0.2 ml. the test dose of toxin and 0.5 unit of antitoxin.

Cl. oedematiens (Bacillus novyi). An anaerobic spore-forming organism about same size as *Cl. welchii*; pleomorphic; Gram-positive (except in old cultures); numerous lateral flagella, but usually non-motile; spores are subterminal. Liquefies gelatin; saccharolytic. In litmus-milk medium forms loose acid clot which sinks to bottom of tube. Forms an exotoxin in fluid media.

Gas-Gangrene Antitoxin (oedematiens). *Standard, B.P.* The standard preparation is a quantity of the dried gas-gangrene antitoxin (oedematiens) kept in the National Institute for Medical Research, Hampstead.

Unit. The unit is the specific neutralising activity for gas-gangrene (oedematiens) toxin contained in such an amount of the standard preparation as the Medical Research Council may from time to time indicate as the quantity exactly equivalent to the unit accepted for international use.

Method of Comparison. The following is an abbreviated account of the method suggested in the *B.P. Add. I*. A test toxin is prepared by growing *Clostridium oedematiens* for about five days; the medium is sterilised by filtration and ammonium sulphate is added until a precipitate is formed which is collected and dried. The test dose of the test toxin is determined in one of two ways, either by intramuscular injection into mice or intracutaneous injection into guinea-pigs. The test dose is the least amount of toxin which mixed with 0.02 unit of the standard preparation kills about half the mice receiving it; alternatively it is the least amount of toxin mixed with 0.02 unit of the standard preparation which causes a reaction in the skin of the guinea-pig. When the test dose is determined by one of these methods unknown samples of antitoxin are estimated by the same method. Mixtures are made so that 0.2 ml. contains in normal saline solution 0.1 ml. of the solution of the toxin and different quantities of the antitoxin being tested. The mixture of the antitoxin being tested which contains 0.02 unit in 0.2 ml., is that mixture which kills the same, or most nearly the same, number of the mice as the mixture containing 0.02 unit of antitoxin in 0.2 ml.; alternatively, that mixture which contains 0.02 unit in 0.2 ml. is that which produces the same degree of local reaction in guinea-pigs as that produced by the mixture containing in 0.2 ml. the test dose of toxin and 0.02 unit of antitoxin.

Cl. histolyticum. Anaerobic, spore-forming bacillus, 2-6 μ in length, often in pairs; Gram-positive; oval subterminal spores; strongly proteolytic; in milk media casein is precipitated without coagulation and digestion rapidly follows.

The Permanent Commission on Biological Standardisation of the League of Nations recommended (October, 1935) that the dry stable preparation of *B. histolyticus* antitoxin established by the State Serum Institute of Copenhagen should be accepted as the international standard for this antitoxin and that the unit originally proposed by Weinberg be chosen as the international unit (see *Quart. Bull. Hlth Org.*, L. o. N., 1936, 689). This international unit has been adopted as the American unit. A standard antitoxin for use in the U.S.A. has been prepared and its potency measured in terms of the international standard. One unit of the I.S. antitoxin contained in 0.3575 mg. of the dried serum is equivalent to 0.2556 mg. of the U.S. dried serum. Glycerinated solutions of the U.S. standard are prepared in such a manner that 1 ml. contains 50 units.

Cl. sporogenes. This organism differs from *Cl. welchii* and the *Vibrio septique* in being actively proteolytic, whereas the latter act on carbohydrates rather than on proteins. It is frequently found in putrid wounds and is probably the main cause of their foul odour. Like the gas-gangrene organisms, it is often found in human or animal faeces and in fertilised soils.

It is a bacillus with rounded ends ($5\mu \times 0.8\mu$), actively motile, and Gram-positive; it liquefies gelatin and digests blood serum. It does not seem to be pathogenic but appears to exalt the virulence of the gas bacillus. It is a common contaminant of other anaerobic cultures and, since its spores are highly resistant, it is difficult to separate it from others.

Gonorrhoea. *Neisseria gonorrhoeae* (gonococcus) is a medium-sized diplococcus, 0.8 to 1μ in diameter, reniform in shape, occurring in groups, intracellular in character. It is Gram-negative and morphologically indistinguishable from the meningococcus and *N. catarrhalis*. In films made from the discharge in the earliest stages of the disease, the gonococci are seen in groups of 2 to 20 or more pairs, outside pus cells or overlying epithelial cells, but when the discharge has become purulent they are mostly intracellular. Gonococci are easily killed by drying, but they can stand exposure to cold in the moist state. The organism is very sensitive to temperatures above 37° .

Gonococci ferment dextrose with the production of acid and gas, they do not ferment maltose and mannite.

Diagnosis. In the male, the gonococcus causes an acute urethritis in the first place. There is no difficulty in the recognition of this intracellular diplococcus in properly stained films made from the urethral discharge in the early stages of the infection. In the later stages of infection the gonococcus is not easily found and a more thorough examination is necessary. The following procedure is recommended in chronic cases and particularly when the patient seeks advice as to infectivity when contemplating matrimony. The patient should come for examination in the early morning with instructions not to pass urine till investigated. Films are made from any discharge in the urethra, and the urine passed and examined by naked eye for prostatic threads. The urine should be centrifuged and the deposit stained. The patient should then be placed in the knee-elbow position and the prostate massaged. The prostatic fluid is collected and examined by films and cultures on serum agar. If a purulent morning discharge is absent, if there is no pus in the urine and if the prostatic fluid is clear and contains only mononuclear cells, the patient, in the absence of clinical signs and symptoms, is probably free from gonorrhoea.

The examination of the female genito-urinary tract for gonococci is less commonly successful, even in the acute stage, and greater reliance should be placed on the cultural than on the film results. Before pronouncing a woman free from infection, at least three tests should be made. Films and cultures should be taken from the interior of the urethra, from the cervical canal after passing a speculum, from the vagina, and, if clinically infected, from Bartholin's duct.

The diplococcus can usually be readily found in large numbers in discharges of gonorrhoeal origin, but a diplococcus of similar appearance is also apparently to be found not infrequently in vaginal discharge of non-gonococcal origin. If a distinction is to be made it is best to try to grow the organism in question on the ordinary forms of culture media, since, while the gonococcus will not grow on plain agar, it grows freely on blood agar. On the other hand, the other forms of diplococcus met with in the vagina usually grow freely on plain agar. It is also possible that the presence of the diplococcus inside the pus cell is characteristic of the gonococcus, but one must be a trained microscopist, who is continually examining such preparations to be certain that what appears to be inside the cell is not really lying directly below or above it. Therefore, in cases in which a diagnosis is of serious importance, it should never be based on a mere clinical examination.

Staining. Films made from discharge in a frank case of acute gonorrhoea are characteristic as regards the intracellular position of the gonococci but

diagnosis can *not* be based on presence or absence of intracellular diplococci. For official purposes Gram's method of staining must be used. Incorrect technique, especially over-decolorisation or the use of a strong counterstain may make organisms which are really gram-positive appear gram-negative.

In decolorising, *absolute* alcohol must be used, i.e. 98% or over. *Weak* alcohol decolorises gram-positive organisms. It should not be used for more than 2 minutes.

Jensen's Modification of Gram's Method is rapidly carried out and gives good results.

Make a thin film on a slide by spreading a platinum loopful of pus obtained from inside the urinary meatus, dry with gentle heat and pass rapidly three times through a Bunsen flame; cool. Stain with 0.5% aqueous methyl violet (6B) $\frac{1}{2}$ to 1 minute. Pour off the bulk of the stain and wash away the remainder with a drop or two of strong aqueous iodine solution (iodine 1, potassium iodide 2, water 100). Do not wash off with water. Pour on a fresh quantity of the iodine solution and leave $\frac{1}{2}$ to 1 minute. Wash with *absolute* alcohol till no more colour comes away (maximum time 2 minutes). Without washing in water, counter-stain with:

Neutral Red Solution. Neutral red 1 g., 1% acetic acid 2 ml., distilled water 1000 ml. (made stronger if necessary) for 15 seconds to 1 minute. Wash in water, dry and mount. The gonococci take up the red dye, and appear as rose-pink diplococci, kidney-shaped with their convex surfaces apposed, usually intracellular.

Pyronin Stain, syn. Pappenheim's or Unna's Stain, containing concentrated aqueous pyronin solution 1, concentrated methyl green solution 3, is useful. Stain 5 minutes, wash and dry. Gonococci stain red; cells, etc., blue.

Wyatt Wingrave's Modification = Pyronin (water soluble) 2, methyl green 3, distilled water 100. Dissolve separately, mix and filter. After staining, wash with water and differentiate with 5% resorcin in alcohol.

All organisms, especially the gonococci, stain by this method a brilliant red and pus cells greenish-blue. The gonococci are found in regular clumps of diplococci, the distance between each pair being much the same. Some are intracellular.

A rapid method of examining urethral discharge for gonococci. The film is treated as in Gram's method up to the end-point of decolorisation. Then instead of a red counter-stain, Unna-Pappenheim stain is left on for two minutes and then quickly washed off. Gram-positive and gram-negative organisms will be stained purple and pink respectively; the cell-nuclei however will be bluish instead of pink. The gram-negative organisms are thus more easily detected than when the nuclei are also pink. The Unna-Pappenheim stain consists of methyl green 0.15 g., pyronin 0.5 g., alcohol (95%) 5 ml., glycerol 20 ml., phenol in water (2%) up to 100 ml.—B. R. Sandiford, *Brit. med. J.*, ii/1937, 1151.

Acid Thionin. Thionin 0.5%, glacial acetic acid 5% in distilled water. Stain 3 minutes, wash in tap water. A very reliable stain—shows phagocytosis well and the characteristic "kidney" shape of the cocci. Best stain for general use when confirmed by "Gram."—Wyatt Wingrave.

Nissl's Stain. Methylene blue, "B. Patent." 3.75, soft soap 1.75, water 1000. Stain thin smears (fixed in air) without heating, for 1 minute, wash, blot and examine.

Cultivation. The gonococcus requires blood or serum for growth; suitable media are agar containing 10% of blood, serum-agar prepared from fresh sterile serum, or agar containing 10% of hydrocele fluid. The media must be kept moist during incubation and pH should be adjusted to 7.5. Gonococcus grows aerobically at 30°-39° (optimum 36°-37°); some strains require 5% CO₂ in the atmosphere.

The most satisfactory medium for cultivation of gonococci in a small laboratory is the desiccated Bacto-proteose No. 3 Agar (Difco). It should be enriched just before pouring by the addition of 1/20th to 1/10th its volume of sterile defibrinated blood of horse, sheep or rabbit (in that order of preference) or sterile ascitic fluid, or if none of these is available Bacto-hæmoglobin. Many workers advise heating the blood to a dark brown. To do this, the melted agar may be allowed to cool to 70°-75°F., the blood added, mixed and maintained at that temperature about 10 minutes or until it looks like chocolate, then poured into petri dishes.—*J. Amer. med. Ass.*, ii/1941, 2826.

Complement Fixation Test. In acute complicated cases of gonorrhœa the complement fixation test is strongly positive, while in chronic cases of specially long standing, the reaction is sometimes only weakly positive. Consequently + and ++ reactions must be regarded suspiciously. The test is an aid in the determination of cure if previously positive reactions become negative, and the clinical and bacteriological findings are also negative. The negative reaction by itself does not mean the absence of gonococci. A positive reaction persisting longer than one year after a clinical and bacteriological cure is a strong indication that a latent focus of the gonococcus is present. The test is not intended to replace the clinical and bacteriological methods of diagnosis but should be a supplementary procedure.—A. Cohn, *J. Lab. clin. Med.*, 1937, 23, 627.

It has been proved that smears, even by differential staining, invite an error of over 50%; over 65% of cultures are accurate when an efficient medium is employed; the complement fixation test is nearly 80% correct when Price's improved technique is used. This reaction gives the highest number of positive results; its value is as great in the diagnosis and in assaying the effect of treatment of gonorrhœa as the Kahn and Wassermann reactions are in the management of syphilis.—T. Anwyl-Davies, *Med. Annu.*, 1937, 179.

The complement fixation test is an important diagnostic aid in the routine study of arthritic patients. Of 125 tests done on the serums from 74 cases of proved or probable gonorrhœal arthritis 81.6% gave positive reactions at some time during the course of the arthritis. Of the tests done on the serums from the 52 proved cases of gonorrhœal arthritis 80.7% gave positive reactions at all times. Of 239 cases representing other types of arthritis 91.6% gave consistently negative reactions.—C. F. Warren, W. A. Hinton and W. Bauer, *J. Amer. med. Ass.*, 1/1937, 1241.

Price's Antigen is prepared from a 24 to 48-hour culture of the gonococcus grown on hydrocele-agar. Wash off the culture with 100 ml. of normal saline add 1 ml. of N/1 NaOH and keep in a water-bath at 37° for two hours when, most of the organisms dissolve. Filter through sterile lint, add 1.5 ml. N/1 HCl to the clear filtrate, replace in the water-bath for 10 to 20 minutes, centrifuge at high speed and suspend the deposit in 4 ml. of normal saline. Add N/10 NaOH drop by drop until pH is 7.5. Shake vigorously to dissolve the suspension. Add 1 ml. of 1% formol saline and filter through lint. Place the solution in ampoules of 5 ml., heat at 56° for 2 hours. This colloid fluid constitutes the antigen, and is used in the test in a dilution of 1:30. Must be titrated before use. (See I. N. O. Price, *L.C.C. Monograph*, No. 2995; also *J. Path. Bact.*, 1932, 35, 635).

Guinea-worm Disease, syn. DRACONTIASIS, is due to infestation with *Dracunculus medinensis*, the intermediate host being a cyclops which is swallowed in the drinking water. The disease occurs in the western half of India, in Tropical Africa, and in Persia. The female worm is from 30 to 120 cm. long and about 1.5 mm. in diameter. It inhabits the subcutaneous and interstitial tissues and takes from 9 to 12 months to reach the skin, forming a blister at the point of emergence. This blister later ulcerates and on contact with water permits the reflex discharge of embryos from the prolapsed uterus which perforates the base of the ulcer. Normally it takes about 3 weeks for the worm to empty the uterine contents. During the period of incubation no signs or symptoms are present but just before the worm emerges from the tissue reactions of an anaphylactic nature, such as urticaria, nausea, vomiting, or dyspnœa appear. The lower extremities are usually involved and less frequently the arms, trunk, buttocks and scrotum. When the worm is unable to reach the surface of the skin it dies and may produce an abscess or become calcified giving rise to neuritis or muscular rheumatism.

Treatment. The anaphylactoid symptoms are best treated by injections of 10 m. of 1 in 1000 adrenaline solution. The worm may be removed either

by traction and massage or by multiple incisions under local anæsthesia. The worm may first be killed before removal by the injection of antiseptics such as 1 in 1000 mercuric chloride or 1 in 1000 acriflavine. The outline of the worm becomes more obvious if the tissues are sprayed with ethyl chloride. When the worm is convoluted the whole area may be excised.

Guinea-worm infection may be avoided by boiling all drinking water or filtering through a fine muslin.

Excellent results in 59 cases from injection of phenothiazine. The method of preparation of the injection and the special technique of injection described must be closely followed.—M. Elliott, *Trans. R. Soc. trop. med. Hyg.*, 1942, 35, 291.

Influenza. Epidemic influenza in man is caused primarily by a virus infection, and it is probable that in certain cases this infection facilitates invasion of the body by visible bacteria (see p. 913), giving rise to various complications. A disease was produced in ferrets by the intranasal instillation of filtrates of throat-washings from influenza patients (W. Smith, C. H. Andrewes and P. P. Laidlaw, *Lancet*, ii/1933, 66). The disease is transmissible in ferrets, the infective agent being recovered serially from the nasal passages of sick ferrets only. Throat-washings from healthy persons and influenza convalescents caused no illness in ferrets, nor did the nasal secretions of a subject with a severe common cold. After recovery, the animals were immune to further attack. Human sera, especially from influenza convalescents, were found to contain antibodies capable of neutralising the virus of the ferret disease. Swine influenza virus was also found to cause a disease in ferrets indistinguishable from that produced by human virus.

Since the work of Smith, Andrewes and Laidlaw, most of the more widespread influenza outbreaks throughout the world have yielded a similar virus when it has been looked for, but there has been increasing evidence that apart from sporadic cases and local outbreaks of clinical influenza, definite epidemics may occur in which this virus is absent, or plays only a minor role. In short, it has become evident that the clinical picture historically recognised as influenza is not a single ætiological entity. Confusion may thus be introduced by attempting to confine the term "epidemic influenza" to the disease associated with the presence of that virus, and workers at the National Institute for Medical Research, London, and at the Laboratories of the Rockefeller Foundation, New York (*Lancet*, ii/1940, 413), suggested the adoption of a more precise terminology, as follows:—

Clinical Influenza. An ætiologically indefinite symptom-complex including the symptom-complexes known as "febrile catarrhs."

Influenza A. A specific disease entity caused by infection with any one of the various strains of the virus discovered by Smith, Andrewes and Laidlaw and hereafter to be termed influenza A virus.

The separation of influenza A still leaves a disease or group of diseases resembling it, but as yet of unknown ætiology, and it was suggested that if and when hitherto undescribed viruses were

isolated from this group and were shown to be of aetiological significance, other specific diseases in the group could be labelled influenza B, C, etc., as they were found to be caused by the hypothetical influenza B virus, C virus, etc.

Influenza B. Subsequently, Francis (*Science*, 1940, 92, 405) recovered a new type of virus from epidemic influenza, which he designated "Influenza B Virus," from the throat washings of patients near New York with influenza in February and March, 1940, and its presence was also confirmed in epidemic influenza in the South Eastern States at about the same time.

Several workers have recorded serological differences amongst influenza A viruses, but there is always an unmistakable antigenic overlap. B virus, on the contrary, is serologically quite unrelated to A virus. B virus will infect ferrets and mice, but both the primary transfer from man to ferret and the subsequent infection of mice from ferrets are more difficult than with A virus. Only one strain of B virus has been securely established in ferrets and mice. Sera from patients convalescent from outbreaks of influenza B have shown a specific rise in neutralising antibodies against B virus and not against A virus.

Evidence as to the prevalence of B virus is based on serological evidence rather than on actual isolation of the virus. On this basis an epidemic studied by Francis in California in 1936 was probably caused by influenza B virus, and it appears also that the same virus was confirmed in an outbreak in the West Indies in 1940.—*Lancet*, i/1941, 114.

Outbreaks of infection of the upper respiratory tract in schools, hospitals and service institutions in England between January and April 1939 were investigated. Many cases resembled clinically those from which influenza virus was recovered in the 1937 outbreak. Garglings obtained from patients in January 1939 did not yield influenza virus by inoculation of ferrets; in February and March 1939 only in 12% of cases was the virus isolated and serological evidence of infection with influenza virus was obtained in 33% of cases only. There were no clinical differences between cases in the 1939 outbreaks which could or could not be classified as epidemic influenza on the basis of laboratory tests. There are reasons for suspecting that in 1939 at least two clinically similar diseases were prevalent—one of them being epidemic influenza as studied in 1937.—C. H. Stuart-Harris *et al.*, *Lancet*, i/1940, 204.

No evidence of the presence of influenza A virus was obtained in material from the respiratory infections prevalent in England in the early months of 1940. In January and February 1941 no widespread influenza epidemic occurred in England, but laboratory studies indicated that a considerable proportion of the sporadic clinical influenza prevalent in those months was due to influenza A virus. The viruses infected ferrets less easily than those of other years and were less readily adapted to infect mice.—C. H. Andrewes *et al.*, *Lancet*, ii/1941, 387.

Influenza A virus was causally related to 50% of cases studied in 1940 and 1941 in all but 3 outbreaks. Virus B was found in only 6 cases, 4 of which had also evidence of virus A. Many cases clinically indistinguishable from those associated with virus gave no indication of the presence of either virus. These cases (30%) occurred during a virus outbreak and are due to agents yet unknown, but distinct from influenza A or B virus. The presence of virus A or B was sought in washings from the nose and throat, by inoculation of ferrets and Syrian hamsters; 10 to 14 days later they were bled from the heart and the serum was tested against influenza A or B virus.—E. H. Lennette *et al.*, *Publ. Hlth Rep., Wash.*, 1941, 56, 1777; *J. Amer. med. Ass.*, ii/1941, 1920.

Along with the lesser A outbreaks there occur regularly many cases of clinical influenza which yield no laboratory evidence that A or B viruses are concerned. American workers refer to these for convenience as "*Influenza Y.*" There is a tendency to interpret recent findings as meaning that epidemics are caused by numerous different viruses only two of which have yet been

recognised; further research will probably show that there are more mixed epidemics than would be expected if the causative agents were wholly distinct and independent. "Influenza A" is only prevalent in Britain every other year. The virus has in a few instances been recovered from garglings of healthy persons during epidemics and there is good serological evidence that sub-clinical infections occur during epidemics. These may apparently act as sources of spread of infection. But where is the virus between epidemics? There is no good evidence of recovery of virus from carriers then. Further between one epidemic and another the antibody level of the community seems steadily to fall; in fact the virus seems to disappear for about 21 months out of the 24. A similar position arises in connection with swine influenza, which breaks out in herds of pigs in the Middle West every winter, the virus apparently disappearing every summer. A clue to the mystery in this case has been afforded by Shope's observation that swine influenza virus survives in lung-worms which are almost universally present in pigs' lungs in the Middle West; earth-worms ingest the infected ova and are then eaten by the pigs. It seems very likely that human influenza viruses can also exist in an occult form; not necessarily in a worm; not necessarily outside the human being. It is possible that there may be a gradation virus (i.e., one stripped of a number of its properties, including all or almost all that A-antigen which makes it recognisable serologically as "A-influenza" virus) to pandemic virus. In winter-time a certain number of people may be carrying this avirulent basic influenza virus. The seasonal coughs and colds permit the spreading of this virus and passage through a series of unduly susceptible people allows it to increase its grade of virulence until it can cause symptoms in man. Just as passage through very susceptible persons may lead to an increase in grade of virus, so passage through those relatively immune would be likely to induce a fall, perhaps more rapid than the rise. Here, maybe, lies the explanation of the excessive numbers of "Y" influenzas in people with high antibody-titre. The difficulty is that if virus in such persons rapidly became degraded and lost its A-antigen, we could not detect that virus was present at all; we should merely fail to infect animals, fail to detect an antibody-rise in the patient and label him "Y" or "Influenza of unknown origin."—C. H. Andrewes, *Proc. R. Soc. med.*, 1942, 36, 1.

Incidence. A large increase in the number of deaths ascribed to influenza occurred in the 122 great towns of England and Wales during the last three weeks of December 1936, the figures being 57, 97 and 325. Judging by the number of influenza deaths in the first quarter of 1937, the epidemic of 1936-37 ranks fifth in order of the severity of the death-rate since 1920. The high death-rate was due to excessive prevalence and not to any increase in virulence.—J. D. Rolleston, *Med. Annu.*, 1939, 256.

In 1938 the total number of deaths from influenza in England and Wales was 4847, of which 2077 occurred in the first quarter of the year. In the first quarter of 1939 there were 6213 deaths.—*Rep. med. Offr Minist. Hlth, Lond.*, 1939.

A four-year cycle of major influenza epidemic peaks has been recently prevalent in England. Peaks in 1929, 1933 and 1937 were interspersed with minor prevalences in 1931, 1935 and 1939 and almost nothing in the years adjacent to the peak years. If the cycle had continued, 1940 should have been a year free from influenza and so the laboratory studies indicated; 1941 should have been a peak year, but this did not prove to be the case; the deaths from influenzal pneumonia remained at a fairly low level. The influenza A virus present in England in 1941 was of low infectivity and low virulence.—C. H. Andrewes *et al.*, *Lancet*, ii/1941, 387.

Cultivation. Influenza virus can be cultivated on the chorio-allantoic membrane of the developing chick. The virulence of the virus for the chick embryo is at first low, but is increased by passage. The original antigenic characters of the virus are maintained during cultivation. After repeated culture in the developing chick, cultures can be obtained in a chick-embryo medium *in vitro*. The original inoculum for egg passage is usually a suspension of infected animal tissue containing the virus in considerable quantity. The virus has been isolated by direct inoculation of eggs with naso-pharyngeal washings without preliminary animal passage.—T. Francis and T. P. McGill, *Proc. Soc. exp. Biol.*, N.Y., 1937, 36, 134.

Infection of chick embryos by the amniotic route with material of direct human origin.—F. M. Burnet, *Brit. J. exp. Path.*, 1940, 21, 147 and *Australian J. exp. Biol. Med. Sci.*, 1941, 19, 39.

Identification. A new way of identifying the influenza virus is suggested by the discovery that fluids containing influenza virus specifically agglutinate fowl's red cells. This agglutination, which is apparently produced by the virus itself, is inhibited by the presence of antiviral serum. The immunologically distinct influenza viruses A and B and that of swine influenza can be sharply separated from one another by this simple *in vitro* test which can be read in an hour. The agglutination-inhibiting antibodies appear to parallel the virus-neutralising ones.—G. K. Hirst, *Science*, 1941, 94, 22; and *Lancet*, ii/1941, 283.

Transmission. The influenza virus can withstand drying and may remain alive for days or weeks at ordinary temperature. In an experiment in which a blanket was impregnated with a suspension of influenza virus (PR 8 strain), it was found that as much as 10% of the virus survives drying under ordinary atmospheric conditions and can be distributed in the air on dust particles by shaking the blanket. After 3 days there may be little or no appreciable loss of virus; thereafter there is a steady decrease, between 1 and 10% remaining after a week. Minute dried particles containing influenza virus (droplet nuclei) such as are distributed by coughing or sneezing can become attached to material such as a sheet and persist so that 10% may remain alive at the end of a week. Articles that have been contaminated either directly or indirectly by influenza patients may be infective, for the virus may reach a fresh host either by direct contact or by aërial transfer of dried virus on dust particles. Virus has been demonstrated on dust exposed near a ferret suffering from influenza, but owing to the low infectivity (for animals) of human strains of virus a similar demonstration could not be made on dust in the neighbourhood of infected human patients. Destruction of the virus is much more rapid at 37° and in the light.—D. G. Edwards, *Lancet*, ii/1941, 664.

Hæmophilus influenzae (*Syn. Bacillus influenzae*, Pfeiffer's Bacillus). A very small bacillus, non-motile. Does not stain by Gram's method, nor grow on ordinary media unless hæmoglobin be present. Must now be regarded as only a secondary invader in epidemic influenza.

The pneumococcus occurs very frequently in conjunction with the influenza bacillus. A mixed flora in the secretions in these cases is characteristic. Influenza bacilli are commonly found in the throat in pertussis, measles, and pulmonary tuberculosis. May be found in respiratory catarrhs apart from influenza; it may also be present in normal healthy persons.

Cultivation. *H. influenzae* grows best on agar containing heated blood.

Blood Agar, made by boiling the agar medium with blood for a minute and separating the coagulated protein, is a good medium for growing *H. influenzae*. Or blood 1 ml. may be diluted with 9 ml. of water and boiled. The clear liquid added to nutrient agar is also an excellent medium for the organism, or strong mineral acids, e.g., sulphuric, may be used without heat to act upon blood, and the liquid subsequently neutralised with soda.

Fleming recommends the use of penicillin to facilitate the isolation of *H. influenzae* from sputum, etc.; placed on the surface of the culture medium penicillin inhibits the growth of various gram-positive cocci but permits the growth of *H. influenzae*.

Paul Fildes' Medium. Mix in the following order normal saline solution 150 ml., hydrochloric acid 6 ml., defibrinated sheep's blood 50 ml., and pepsin B.P., 1 g. Shake to dissolve and place in water-bath at 55° for 2 to 3 days. Adjust the reaction to pH 7·6 by adding 20% sodium hydroxide solution 12 ml. or more until a permanganate colour is produced with cresol red. Now add hydrochloric acid drop by drop until cresol red gives practically no change in colour but phenol red gives red (pH 7·0 to 7·2). Finally, add 0·25% chloroform. For use, add the medium directly to melted agar at 60° in a strength of 2 to 5%, or to broth in the same proportion. It is not necessary to remove the chloroform. The correct adjustment of the reaction is important.

Leishmaniasis. Leishmaniasis is the term applied to a group of diseases caused by parasites of the genus *Leishmania*. Some of these are general infections, others are local. The first is represented by kala-azar, due to *L. donovani* which is spread by the blood stream and invades all tissues of the body except those of the nervous system. An infantile form of the disease, Mediterranean kala-azar, has, in the past, been ascribed to *L. infantum*.

Both morphologically and serologically they are the same parasite and there seems no reason to separate them. Oriental sore or cutaneous leishmaniasis, due to *L. tropica*, which is localised to the skin and not spread by the blood-stream, and muco-cutaneous American leishmaniasis (espundia), due to *L. braziliensis*, are, however, distinct species belonging to the second group and are separable serologically.

Kala-azar. *Syn.* VISCERAL LEISHMANIASIS, DUM-DUM FEVER, BLACK FEVER. Occurs mainly in India, particularly in Assam, and in China. The disease is characterised by enlargement of the spleen and liver, anæmia, emaciation, and irregular fever of long duration. The name of "black fever" has been given to the disease owing to the increased pigmentation of the skin to which it gives rise. The parasites develop in the gut of the sand-fly (*Phlebotomus*) but the exact mode of infection is uncertain. The mortality rate in the untreated disease is about 90%.

An infantile form of kala-azar, which has a similar symptomatology to the Indian form, occurs in all the countries bordering the Mediterranean, and is usually found in association with the disease in dogs, the dog-flea, *Ctenocephalus canis*, being the probable transmitting agent. There are, however, areas in which the disease is common in dogs but human kala-azar does not occur. In the Mediterranean area the disease is almost entirely confined to infants under 5 years of age, whereas in India and China most cases occur between 5 and 15 years of age. Canine infection is rarely noted in India and China.

The Mediterranean visceral leishmaniasis was regarded as an infantile disease, whereas the Indian kala-azar occurs amongst people of all ages. But, for some time, it has been found that numerous cases of visceral leishmaniasis exist amongst grown-up people in North Africa, Spain, France, Italy, Sardinia, Sicily. Canine visceral leishmaniasis is constantly present in endemic foci of Mediterranean visceral leishmaniasis, and wherever sufficient investigations have been made, e.g., Algeria, Tunis, Malta, Catania and Canca, it has been found that the disease is commoner in dogs than in human beings. It is probable that the epidemiology of the disease in dogs, as in man, has been constant over a long period. It is practically certain that the causative agents of the human and canine disease are identical.—E. Sergeant and S. Adler, *Quart. Bull. Hlth Org. L.o.N.*, 1935, 806.

Morphology. The rounded non-flagellate stage of the parasite is a small oval body 2 to 5 μ long by 1 to 2 μ broad, containing two structures, one a large round laterally placed nucleus staining bright red with Romanowsky's stain, the other, which is usually rod-shaped, stains a deep reddish-purple and has one end pointing towards the nucleus. It can be cultivated on rabbit-blood agar (N.N.N. medium) if grown for 2 or 3 weeks at room temperature. During growth leishmania bodies develop into leptomonads measuring up to 24 μ in length with a flagellum and centrosome at one end and a central macronucleus.

Diagnosis. Diagnosis is generally dependent on the demonstration of *L. donovani* in smears from the liver or spleen, or cultures on N.N.N. medium obtained from the peripheral blood. Examination of thick films of blood, stained by Leishman's or Giemsa's stains, should give 60 to 70% of positive results in competent hands.

Cultivation. Blood cultures should give 100% positive results if a satisfactory technique is employed; the method is slow, positive results may be obtained within 1 week, but cultures should not be discarded as negative under 1 month. The blood should be taken from a vein in an oil-sterilised syringe; 0.5 ml. added to 10 ml. of 0.85% saline with 2% sodium citrate. The cells are

allowed to settle and are sown into N.N.N. tubes, kept at 22°; drops of the condensation fluid are examined at intervals. The flagellates will be seen as actively moving forms among the red cells. As the medium is easily contaminated, it is advisable to sow at least three tubes.—L. E. Napier, *Brit. Encyclop. Med. Pract.*, 7, 354.

N.N.N. Medium (Novy, MacNeal, Nicolle). Take 14 g. of agar, 6 g. of sodium chloride and 900 ml. of water. Prepare as for ordinary agar, pour into tubes and sterilise. To one part of this medium liquefied and cooled to 48° add one-third its volume of defibrinated rabbit's blood. Mix thoroughly, slope and allow to set. Use rubber stoppers to prevent evaporation of the condensation fluid.

Noeller's Modification of N.N.N. Medium. Agar 25 g., glucose 20 g., broth (slightly alkaline) 1 litre. When required for use melt and add twice the volume of defibrinated horse blood, or non-defibrinated rabbit blood.

Cultures of leishmania of oriental sore and kala-azar can be differentiated by the type of growth on blood agar plates (Noeller's medium). In the case of *Leishmania tropica* a streak inoculation gives in the course of a few weeks a growth which is characterised by off-shoots, sometimes branched, from the main streak, while *L. donovani* gives a simple streak without side outgrowths.—M. Mayer and B. Malamos, *per Trop. Dis. Bull.*, 1937, 43.

Vital Staining. Culture forms of leishmania taken from the surface of a medium and suspended in physiological saline solution may be vitally stained by placing a drop of the fluid on a slide on which an alcoholic solution of brilliant cresyl blue has been allowed to evaporate. After staining, the flagellates in the fluid are exposed to the vapour of osmic acid and then spread on to a film and dried. The film is then stained by Romanowsky stain. The nucleus is seen to have a violet red margin of chromatin and a blue central karyosome. In the cytoplasm are masses of a substance resembling the crinome of *Chlopin*. Between the masses the pellicle, stained a violet colour, is seen. It is concluded that the masses consist of a substance which in life is uniformly distributed through the cytoplasm and that, having a negative charge, it is flocculated by the basic dye.—Ch. Mochkovski, *C. R. Soc. Biol., Paris*, 1936, 1607.

Aldehyde Test. To 1 ml. of clear serum add 1 or 2 drops of commercial formalin. In a strongly positive case the serum becomes absolutely solid and completely opaque like the white of hard-boiled egg within a few minutes; if it becomes completely opaque in 24 hours the result is still positive. In a negative result the serum remains crystal clear, although it may solidify. Doubtful results are solidification with various degrees of cloudiness. The reaction is seldom completely positive after 1 month from the onset of the disease; after 5 months it is always completely positive and may be taken as absolutely diagnostic of kala-azar.

Chopra's Antimony Test. 1 ml. of serum is diluted with ten times its volume of distilled water in a narrow-bore tube; add 4% solution of Urea-Stibamine from a Wright's pipette and mix by rotation. A strongly positive reaction is shown by a heavily flocculent precipitate forming immediately and settling as a flocculent mass in half an hour. A fine granular precipitate, settling more slowly but forming a compact mass at the bottom of the tube is a doubtful positive. No precipitate denotes a negative. The reaction is said to be more delicate than the aldehyde test and is usually positive within two months from the onset of the disease.

Treatment. The specific treatment for kala-azar is the intravenous administration of antimony, usually in the form of one of the pentavalent compounds (see Vol. I).

Oriental Sore. *Syn.* CUTANEOUS LEISHMANIASIS, ALEPPO SORE, DELHI BOIL, BAGHDAD BOIL, BISKRA BUTTON, ETC. This condition has a wide distribution, occurring in the north-western parts of India, Arabia, Iran, Asia Minor, Mesopotamia, North Africa, Spain, Italy, and Greece. It commences as small itching red papules, developing into nodules which break down and form chronic indolent ulcers, the ulcers having well-defined rounded edges and a granulation tissue base exuding thin pus. It is produced by *L. tropica*, the organism occurring in the thickened edge

of the sore. The transmitting agent is a fly, probably *P. papatasi*. The condition is practically never fatal but if left untreated it may last for 18 months, after which the patient is usually immune to further infection.

Diagnosis. This can only be made with certainty by the finding or cultivation of the parasite. The parasites may be found in pus from the ulcer surface, but are more readily discovered by puncture of the unulcerated margin. Direct microscopic examination of this material often shows *L. tropica* in the endothelial cells. Cultures may be made on N.N.N. medium.

The condition must be distinguished from the so-called trench sores, due to staphylococcal infection, and from tropical ulcer; rodent ulcer, extra-genital chancre and post-visceral dermal leishmaniasis are other conditions which may simulate oriental sore.

Treatment. Oriental sore must no longer be regarded as a local disease, and a vaccine from *L. tropica* has been used with encouraging results. Berberine sulphate should never be given where there is sepsis or inflammation. Sores may be classified into two types—and 1% and 2% solutions of berberine should be used respectively. The maximum amount of solution which may be injected is 4 ml. of a 1% solution and it is not considered necessary to give a local anæsthetic prior to infiltration. Intravenous injections of tartar emetic solution have so far given better results than have any other organic antimony preparations.—Warma, *Indian med. Gaz.*, Nov., 1934.

Antimony in either its trivalent or pentavalent form is still the most effective and cheapest drug in the treatment of oriental sore. The dose of potassium antimony tartrate should be regulated according to the weight of the patient and should never exceed 2 gr. at one time. It must be carefully injected owing to the sloughing it causes if allowed to escape into the tissues. Berberine is not as successful as might be expected from the results claimed for it; for single sores or small lesions, before they have broken down, it gives good results but is disappointing with large or multiple sores. Early treatment is most essential; the recently developed case is comparatively easy to cure. Dressings of gauze soaked in normal saline should be changed twice daily.—J. Goodall, *Indian med. Gaz.*, 1937, 3.

Favourable results obtained from the application of Cignolin paint to the sores, with rapid healing in the particularly intractable indurated sore on the nose. The paint should be applied daily to the sore for a period of fourteen days or longer. The method is simple and painless.—P. Manson-Bahr, *Med. Annu.*, 1937, 267.

Solution of Atebrin injected into the skin round oriental sores rapidly destroys the parasites and cures the disease. The dose administered at one sitting commences at 0.05 to 0.1 g. in 1 or 2 ml. of distilled water and is increased at subsequent sittings to 0.3 g. In some cases only a single injection is required to bring about a cure.—F. Flarer, *Trop. Dis. Bull.*, 1939, 454.

Espundia. *Syn.* AMERICAN DERMAL LEISHMANIASIS, UTA, PIAN BOIS, FOREST YAWS, BOSCH YAWS, ETC. Espundia has a wide distribution in the tropical parts of South America and resembles oriental sore. It produces ulcerating granulomatous lesions of the mucous membrane of the nose and throat. The condition is due to infection with *L. braziliensis*, *P. lutzi* being suspected as the transmitting agent. Untreated cases are liable to die of intercurrent disease or cachexia, but the condition responds well to antimony treatment.

Leprosy, a disease which is prevalent in many tropical and sub-tropical countries, is due to infection with *Mycobacterium lepræ* (Hansen's bacillus), and is associated with characteristic lesions involving the skin and mucous membranes (nodular type) and the nerves (anæsthetic type). It is not hereditary and cannot be considered a highly contagious disease since intimate

contact with lepers is essential to its contraction and only 3% of people living with lepers contract the disease. The nodular cases with numerous bacilli are more infective than the nerve cases with very few organisms. Children in the first two or three years of life are more susceptible to infection than adults. The incubation period may vary from a few weeks to many years. The primary lesions are most frequently seen on the extensor surfaces of the limbs and on the more exposed parts of the body. There is little evidence that the organisms are inoculated by insects and recent evidence is against primary nasal infection. Syphilis, presence of intestinal parasites, debilitating diseases, improper diet, and long residence in hot, moist and enervating climates are potent predisposing causes.

Leprosy is not a fatal disease. In Northern India the majority of clinically diagnosed cases never progress to the more serious forms of leprosy and cause little or no physical inconvenience. The important determining factors in making a prognosis in leprosy may be summarised as follows:—(1) The degree of natural resistance, which may possibly vary in individuals. (2) The age factor; the resistance to leprosy infection is low during the first few years of life. (3) The general health; the more chronic and non-toxic an infectious disease is, the greater relative importance does this factor assume. (4) Depressed immunity due to hyperinfection. (5) Acquired immunity as the result of small (subliminal) infections.

The Lepra Reaction is one of the best known phenomena in leprosy. The clinical signs are a general febrile condition, swelling of former apparent lesions and the appearance of lesions where they were not formerly visible. Hollman states that the reaction is not a fresh manifestation of the disease but an inflammatory reaction in previously unrecognised foci. Bacillæmia is present, at least in the more advanced skin cases. In neural leprosy the involved nerves become swollen and painful. Leprosy reaction may occur apparently spontaneously, but is generally associated with some condition that lowers the general health; it may also be caused by treatment. For the sake of convenience two types may be designated: (a) that which is of a toxic nature and subsides when the cause disappears or is removed by treatment, and (b) that in which, in addition to the presence of toxæmia, the patient has become sensitised and the reaction tends to continue indefinitely or to recur at intervals. The former type may pass into the latter unless precautions are taken. Excessive treatment may produce reactions of the second type. It is well known that leprosy reaction has the effect of causing granulation, or breaking up of the bacilli into acid-fast clots.—E. Muir, *Int. J. Leprosy*, 1933, 432.

Distribution. It is impossible to form even an approximate estimate of the number of sufferers from leprosy in the world, but the following table gives maximum and minimum figures between which actual truth probably lies.

| | Minimum | Maximum |
|---|-----------|-----------|
| China | 1,000,000 | 1,500,000 |
| India | 500,000 | 1,000,000 |
| Africa | 500,000 | 1,000,000 |
| British Empire (excluding Africa and India) | 15,000 | 30,000 |
| South America | 100,000 | 150,000 |
| Europe | 6,000 | 10,000 |
| Other Countries | 100,000 | 150,000 |
| Total | 2,221,000 | 3,840,000 |

Roughly from 2 to 4 millions in the world.—*Brit. Encyclop. Med. Pract.*, 7, 683.

Morphology. *Mycobacterium lepræ* (Hansen's bacillus) has a morphology similar to *M. tuberculosis*, but usually occurs more in clumps and is said to be tapered at the ends. It stains irregularly, and is more readily decolorised than *M. tuberculosis* by inorganic acids.

Cultivation. Attempts to cultivate *M. lepræ* have been made by many workers, but the positive results obtained lack confirmation. Cultures of acid-fast organisms have been isolated from leprosy lesions, but it is doubtful if any of these is the true leprosy bacillus.

The successful inoculation of Syrian hamsters with human leprosy. Young animals were splenectomised and the skin in the neighbourhood of the incision was separated from the fascia of the abdominal muscles over a wide area and a fragment of human leprosy nodule was placed in the space between the skin and fascia. The incision was closed with catgut. Later the animal was given an intraperitoneal injection of macerated human leprosy material. After six weeks there was a considerable multiplication of leprosy bacilli around the original implant and some distance from it. Leprosy bacilli were found in smears from the liver.—S. Adler, *Lancet*, ii/1937, 714.

Burnet in 1938 obtained in 1 out of 6 inoculated hamsters what he considered to be definite signs of progressive disease. Dharmendra and Lowe in 1940 repeated the experiments of Adler and Burnet on a larger and more prolonged scale, but failed to substantiate their findings. The only safe criterion of successful inoculation is the passage of the disease through an indefinite series of animals.—*Lancet*, ii/1940, 628.

Diagnosis. In nodular leprosy the demonstration of *M. lepræ* by stained smears is usually easy—in the skin nodules, the nose, the regional glands and even in the blood. In nerve leprosy, however, the organisms are very sparse and in these cases examination of material from the nose may be the only means of establishing the diagnosis, after inducing a drug coryza by giving 60 gr. of potassium iodide. The organisms may be found in the early exanthems that often precede the development of the characteristic features in both types of the disease but not ordinarily in the anæsthetic macules secondary to nerve injury. Smears may be prepared from the scrapings of any ulcerated nodule or from the serum obtained by puncturing a nodule. For staining, the Ziehl-Neelsen method is used, decolorising lightly with 20% aqueous sulphuric acid or 3% aqueous HCl. The bacilli are present in the blood in sufficient number to be demonstrated directly in many cases of nodular leprosy. They may be demonstrated in thick slide preparations as used in malaria; after de hæmoglobinisation they are stained in the usual way.

In leprosy the sedimentation rate of the red blood cells is increased and is a useful index of the progress of the disease.—Stitt.

Histamine Test. When a dilute solution of histamine is pricked into the normal skin a sharply defined circular local reddening appears in about 20 seconds, followed in another 15 to 30 seconds by a flush or flare of a dark red or scarlet colour on the surrounding skin, and later by a discrete weal at the site of the prick. For the diagnostic test of early leprosy a 1:1000 dilution of histamine phosphate in normal salt solution is employed. A small drop of the solution is carefully placed within the suspicious macule to be tested and another dropped on normal skin at least one inch away from the border of the lesion for control. With a sharp pin a prick is made through the drop into the skin underneath, taking care to avoid bleeding. The histamine solution is wiped off immediately and the pricks closely observed, under good, natural light. The test is said to be negative when the complete response is elicited and positive when the flare is absent. The flush is always absent in the depigmented macule of leprosy; the weal in the macule is usually the same size as

that on the normal skin. The test has been applied on macules of other skin diseases which may be mistaken for the pale macule of leprosy, but in every case the flare is present provided the individual is not unsusceptible to histamine. In the case of the reddish macule of leprosy only the weal may be elicited, but when the colour is not so striking the local redness may be elicited, but redness of the original lesion is at all bright it is best to prick the histamine solution just inside the border; in the non-leprotic lesion the flare appears on the adjacent portion of the skin outside the border, whereas there is no such flare extending from the macule in early leprosy.—J. Rodriguez and F. C. Plantilla, *Leprosy Rev.*, 1932, 18.

Pilocarpine Test. The intradermal injection of pilocarpine, 0.2 ml. of a 1 in 1000 solution, is a valuable auxiliary means of diagnosis in early and slight cases. The injection is given into small doubtful lepromatous patches, a second injection being given into normal skin as a control. Tincture of iodine is then painted over an area covering the site of both injections and when this has dried powdered starch is dusted on. The positive inoculation and when this dry but there is sweating for a few millimetres round the control, thus bringing the iodine and starch in contact and forming a blue stain.—E. Muir, *Leprosy Rev.*, 1939, 121.

Leprolin Test. This test, first used by Mitsuda and more generally adapted by Hayashi, is of immense value in testing the degree of natural and acquired immunity and the degree of depressed immunity due to hyperinfection. In the modified form of the test used in Calcutta two leprolins are used: (a) a suspension of ground-up human lepromatous material (*Hansen leprolin*), and (b) as a control a similar suspension of omentum, spleen, and liver of rats suffering from advanced rat leprosy (*Stefansky leprolin*). These two suspensions contain respectively *M. lepræ* and *M. lepræ muris*, mixed with tissue debris. They are sterilised by heat and standardised so that they give equal reactions when 0.2 ml. of each is injected intradermally in human subjects who have not been infected with leprosy. These leprolins when injected into the skin produce within a week or two at the sites of inoculation small nodules having the consistence of a pea, and varying in size with the degree of resistance; the greater the resistance the larger is the nodule formed.

In young children and in those with bad general health the reaction to both leprolins is weak or negative. In those who have acquired resistance to both small infections with leprosy, the reaction to Hansen leprolin is increased and appears stronger than that to Stefansky. Where, however, hyperinfection has taken place and the bacilli have increased in the body, the reaction to Hansen leprolin is weak or negative, though in adults whose general health is otherwise good the reaction to Stefansky leprolin may be as strong as in non-lepers.—E. Muir, *Leprosy Rev.*, 1935, 187.

The isolation of a protein antigen of *M. lepræ*. A skin test employing this antigen in place of the classical Mitsuda test (the leprolin test) was found at least as sensitive, to give results of the same significance, and to have the advantage of giving rapid results with an absence of unpleasant nodules and ulcers.—Dharmendra, *Ind. J. med. Res.*, 1942, 30, 1-22.

Treatment. In addition to the well-known use of chaulmoogra and hydnocarpus oils and their various salts and esters, innumerable other remedies have been tried. Thus, Chopra (*Handbook of Tropical Therapeutics*, 1936) mentions the following as having been employed with some measure of success: Eucalyptol (intramuscularly), tartar emetic (intravenously), gold preparations, creosote (injected in combination with hydnocarpus or chaulmoogra esters), autogenous vaccines, ultra-violet light. The intravenous use of dyes such as methylene blue was advocated a few years ago, but the results were uncertain and the treatment not without danger. The most recent innovation is the use of diphtheria antitoxin or toxoid, and early reports claim encouraging results for this treatment. As general measures, good hygiene, a proper diet and sufficient exercise are of the utmost importance.

The Use of Dyes in Treatment. Various dyes given intravenously; diminution of external lesions after following:—25 ml. 4% trypan blue; 10 to 20 ml. of 1% brilliant green; 10 ml. of 2% fluorescein—too early to say if improvement permanent. Trypan blue and fluorescein together produce marked retrogression of lesions in about 9 weeks.—G. A. Ryrie, *Trans. R. Soc. trop. Med. Hyg.*, June 22, 1933, 33. Subsequent results disappointing.—*ibid.*, *Leprosy Rev.*, 1938, 20.

Methylene Blue. Intravenous injections, combined with chaulmoogra oil treatment, show rapid and continuous improvement in early cases. In cases of old standing the first rapid improvement is followed by a stage of relative stagnation. It appears that methylene blue has an elective affinity for leprosy tissues, the leprosy lesions assuming a blue colour following injections.—Montel, *Lancet*, ii/1934, 891.

At the Brazil Conference on the treatment of leprosy (June, 1935) some six lectures were given on the use of methylene blue and the conclusions reached were very varied. Alongside the very encouraging results obtained, especially the immediate results in the improvement of infiltrated lesions, cicatrization of chronic ulcers, relief of neuralgias and "reactions," there have to be recorded also the late toxicity secondary to high dosage, with certain untoward reactions, asthenia, wasting and even one fatal accident. There was unanimity in recognising the affinity of methylene blue for the diseased tissue, which becomes impregnated with it at the very first injections; there was the same unanimity that those lesions very soon get "disinfiltrated" of the remedy and recur with greater or lesser intensity, especially the tubercles and the infiltrations; but there was no unanimity in admitting any definitely curative therapeutic action as superior to that of chaulmoogra.—*Leprosy Rev.*, 1936, 72.

Fluorescein. When used as an "interval" treatment between courses of iodised esters better results are obtained. The "interval" treatment consists of 4 to 6 injections of a 2% solution of fluorescein, freshly prepared with distilled water, and sterilised by boiling and filtering, given in 10 to 20 ml. doses twice weekly (during an interval of one month). It serves to avert the lepra reaction commonly experienced if specific treatment with esters is pushed to any undue extent.—E. S. R. Alfred, *Leprosy Rev.*, 1935, 179.

Unable to substantiate the good results observed by other workers using a solution: fluorescein 2 g., sodium bicarbonate 2 g., in distilled water 100 ml. But good results in certain forms of acute iritis consecutive to lepra reaction.—J. M. M. Fernandez and S. Schujman, *Leprosy Rev.*, 1935, 182.

Massive treatment with dyes cannot be recommended. Smaller doses do not appear to have a curative effect and doses sufficient to cause evacuation of leproma are apt to be extremely dangerous. It would seem as if extremely concentrated dye in leproma acts as an irritant foreign body and results in its own evacuation, the bacilli and the remainder of the leproma sharing in the process. The action of dyes given in small doses over a short period to control lepra reaction is a different matter; used in this way they are safe and of considerable value.—E. Muir, *Leprosy Rev.*, 1940, 168.

A highly satisfactory treatment for lepromatous ulcers consists in painting the ulcer first with a 1% solution of gentian violet in alcohol, then with a 10% solution of silver nitrate in distilled water, and, lastly, with a 15% solution of tannic acid in water. On the first day this is repeated three or four times, but in most cases after the first day one painting, either with the tannic acid alone, or with the dye solution followed by tannic acid, is sufficient. After a few applications the discharge is controlled, the patients are more comfortable and dressings are unnecessary.—E. Muir, *Leprosy Rev.*, 1941, 40.

Lepromatous ulcers are now treated by the application of a mixture of gentian violet, brilliant green and acriflavine, without any bandage, and with very good and economical results.—C. J. Austin, per *Leprosy Rev.*, 1941, 61.

The Use of Diphtheria Antitoxin or Toxoid in Treatment. One hundred patients were treated with diphtheria toxoid, in increasing doses from 0.5 ml. to 10 ml. spread over a period of 7 months. Of the 72 cases who completed the treatment 12 became worse, 34 showed no appreciable change, 21 showed a slight improvement, and 5 a marked improvement.—A. R. Davison and E. Grasset, *Leprosy Rev.*, 1941, 78.

Encouraging results obtained with diphtheria antitoxin and diphtheria toxoid. In lepromatous cases there was a reduction in the nodules and plaques and a change in pigmentation from red to brown. The effect of the treatment was

less marked in the nerve cases.—D. R. Collier and J. H. McKean, *Leprosy Rev.*, July, 1940.

In over 600 cases treated with diphtheria toxoid or antitoxin results far exceeded those with any other form of treatment. Fifty per cent. of all the early cases treated for six months or more became symptom-free, while the more advanced cases showed definite improvement in a large number of instances.—D. R. Collier, *Int. J. Leprosy*, January, 1941.

Diphtheria antitoxin injected during an exacerbation of the disease resulted not only in a clearing-up of the reaction, but also a seemingly permanent improvement. This was followed by diphtheria formol toxoid with encouraging results. Work carried out at Leprosy Institution at Chengmai, Siam, still in experimental stage. (Report by E. Muir to British Empire Leprosy Relief Association.)—*Med. Annu.*, 1941, 250.

Improvement obtained in neural cases of the tuberculoid type from injections of tubercle endotoxoid. The injections were given subcutaneously bi-weekly commencing with 0.05 ml. and increasing each week until 2 ml. was given at each injection, the treatment continuing for 6 months.—A. R. Davison, *Leprosy Rev.*, 1941, 18.

Fallacies in Evaluation of Treatment. In evaluating the effect of any drug or line of treatment there are various fallacies which have to be guarded against. These are: (a) mistaking the subsidence of lepra reaction for elimination of the disease, and assuming to be an effect of the drug the granulation of bacilli that such reactions cause; (b) crediting the clearing up of leprosy manifestations to a treatment when it is really due to removal of another disease or aggravating factor; (c) ascribing a direct or "specific" effect to the results of protein shock or of counter-irritation by CO₂ snow, trichloroacetic acid, etc. Certain drugs cause apparent improvement by desensitising the patient. Thus red and swollen lesions may become pale and flat and may even disappear. But bacteriological examination before and after fails to show diminution of the infection. This action is associated largely with small doses of the heavy metals such as antimony, arsenic, copper, gold, etc. It is not unlikely that the recent popularity of dyes, such as methylene blue, fluorescein, etc., may in large measure be due to their desensitising effect. Leprosy is a disease of very low toxicity. There is, therefore, nothing inconsistent between suffering from moderately advanced cutaneous leprosy and attaining good general health. Mass treatment and sole reliance upon drugs are likely in the long run only to bring discredit upon anti-leprosy measures.—*Leprosy Rev.*, 1936, 52.

The signs of active leprosy, as defined by the Leonard Wood Memorial Conference, include: positive bacteriological findings in skin or mucous membrane determined by the usual methods; the presence of raised or erythematous lesions; increase or diminution of lesions in size or number; tenderness of nerves with or without thickening. The aim in the treatment of leprosy is to restore the general health of the patient and to remove all active signs of the disease, thereby rendering it "quiescent." Thereafter the patient is to be kept under observation for a period of two years, during which active signs must continue to be absent. Only when the patient has stood this severe test should the disease be declared "arrested."—E. Muir, *Int. J. Leprosy*, 1933, 433.

Potassium Iodide as a Provocation Test. The drug is given once a week in the following doses: 5, 10, 20, 40, 80, 160, 240, 320 and 320 grains in a pint or more of water. In positive cases there are skin reactions, with or without pyrexia. The test should be applied only to those cases in good physical condition and who appear clinically fit for discharge. It is not infallible, but is distinctly useful.—B. Moiser, *Leprosy Rev.*, 1942, 6.

Lymphopathia Venereum. *Syn.* LYMPHOGNANULOMA INGUINALE, CLIMATIC BUBO, PORADENITIS, PORADENOLYMPHITIS INGUINALIS. An infectious disease of world-wide distribution and protean manifestations. The disease is due to virus infection and is communicated venereally. The infection causes the following conditions:—(a) adenitis affecting the inguinal and upper femoral glands; (b) suppurating bubo; (c) elephantiasis of the vulva (esthiomène); (d) fibrous stricture of the rectum. By far the most frequent manifestation is inguinal bubo. The incubation period

varies from a few days to several weeks, but is usually less than one week. The primary lesion is usually on the external genitals, sometimes near the anus and occasionally on extra-genital sites. It consists of a small herpetiform ulcer, usually slight and healing rapidly, but often it is transitory and the inguinal glands appear to enlarge without a genital lesion. The glands are large, discrete, slightly fixed and only slightly tender on palpation. Usually several glands are involved and these may become adherent and a large irregular mass is formed. One or both inguinal regions may be involved and the disease may be more advanced on one side than the other. Resolution may take place in the affected glands but usually after a variable period the glands tend to suppurate and point externally, discharging an odourless pus which is occasionally blood-stained. There is slight systemic upset with irregular low fever. The suppurating bubo discharges for a considerable time—sinuses are formed and fresh glands may soften, suppurate and discharge. The glands eventually subside and the sinuses heal after a very chronic course. Sometimes there is great destruction of the glands and surrounding tissue and elephantiasis of the leg may result. Wassermann and Kahn tests are negative.

Arthritis, meningitis, conjunctivitis, orchitis and skin rashes may follow infection with the virus of lymphopathia venereum.—Favre and Hellerstrom, *Rev. d'Hyg.*, 1939, 61, 401.

The non-gonococcal form of urethritis, known as millet-seed urethritis, is in reality due to the same virus.—Harrison and Worms, *Brit. J. vener. Dis.*, 1939, 15, 237.

Lymphogranuloma venereum is a common cause of proctitis and sigmoiditis with or without stricture formation. Its course may be extremely chronic. The disease may simulate non-specific ulcerative colitis. The presence of lymphogranuloma venereum may be easily established by means of the Frei intracutaneous test, provided the test is properly used and controlled.—W. L. Palmer *et al.*, *J. Amer. med. Ass.*, ii/1942, 517.

Frei Test. This consists of the intradermal inoculation of sterile material derived from the suppurating bubo characteristic of the early stage of the disease, in a patient who has been proved to be free from tuberculosis and from syphilis, gonorrhoea or chancroid, past or present. Blood-free pus is aspirated from a bubo with aseptic precautions. It is tested for the presence of pyogenic organisms, e.g., staphylococci, by culture. The pus is diluted with normal saline solution (1 : 5 or 1 : 6) filled into ampoules and heated for 2 hours at 60° on the first day and for one hour at 60° on the second day. The antigen is then tested again for sterility by both aerobic and anaerobic cultures. The antigen should be stored at a low temperature and protected from light. An intradermal injection of Frei's antigen 0.1 ml. is made on the forearm and a control injection is made with sterile saline at a distance of three inches from the first. A positive reaction is indicated by the appearance within 48 hours of a papular reaction usually with a zone of erythema of one inch diameter. A positive reaction indicates the presence of, or a previous infection with, lymphogranuloma inguinale. It may be obtained many years after infection and may be elicited throughout the patient's lifetime.

For the preparation of the antigen the pus may be frozen and dried *in vacuo*. The dried powder is diluted with 50 parts of buffered saline and sterilised as above.

A brief résumé and discussion of some possible causes of error in the skin test.—W. Frei, *J. invest. Dermat.*, i/1938, 367.

Antigen prepared from lymphogranulomatous mouse brain can replace that prepared from pus from a human being for the diagnosis of lymphogranuloma inguinale. Not only is it as sensitive and specific as the most potent antigen prepared from human materials, but it overcomes the disadvantages of the latter.—A. W. Grace and F. H. Suskind, *Arch. Derm. Syph.*, N.Y., 1936, 34, 65.

Yolk-Sac Antigen (for use in Frei Test). The virus can be propagated in large amounts when inoculated into the yolk sac of the developing chick embryo, and can be separated from much of the yolk and tissue by differential centrifugation. The yolk-sac antigen is prepared by liberation of the virus from the cells of the yolk sacs by grinding with abrasion and suspending the material in saline solution; the virus is separated from the yolk-sac constituents by differential centrifugation and the sediment containing the virus is diluted in saline solution to a volume two hundred times that of the original yolk sacs; solutions of formaldehyde (0.1%) and phenol (0.25%) are added to inactivate the virus and the final product is tested for sterility on culture media and yolk sacs of normal eggs. This antigen is used for the intradermal Frei test in the usual way, using 0.1 ml., and the reaction read after 48 to 72 hours. It is more sensitive than mouse brain antigen.—G. Rake *et al.*, *Amer. J. Syph.*, 1941, 25, 687.

Yolk-sac antigen (lygranum), i.e., antigen prepared from infected yolk sac of developing chick embryo, is superior to mouse brain antigen for performance of the Frei cutaneous test for lymphogranuloma venereum. Comparatively few non-specific reactions are produced with the egg control material as compared with the mouse brain control.—S. E. Sulkin, *J. Amer. med. Ass.*, i/1941, 2663.

The original Frei antigen has disadvantages. The yolk-sac antigen may be successfully employed in the intradermal Frei test carried out in the usual way or it may be used in a complement fixation test. The latter test is more delicate than the Frei test in detecting border-line cases.—*J. Amer. med. Ass.*, i/1942, 538.

Treatment. The early manifestations can be rapidly controlled. The chronic lesions can also be relieved but much slower improvement is observed. Specific treatment by intravenous or intradermal injections of Frei's antigen produces a rise of temperature, malaise and temporary exacerbation of the lesions followed by subsidence in 48 hours. Initial dose is 0.2 ml. and subsequent dosage is determined by the reaction shown and the progress of the lesion. Non-specific protein therapy, e.g., T.A.B. vaccine intravenously, is a valuable method of treatment. A course of 6 injections may be required and initial dose of 50 million organisms. Injections are given at intervals of 3 days, each injection being about double the previous injection. A marked general reaction with rise of temperature to 103°F. is desirable. Dmelcos vaccine is also valuable, and antimony injections have a slight beneficial action.—Robert Lees, *Practitioner*, ii/1936, 179.

Nine out of eleven patients obtained complete cure from repeated roentgen irradiations in small doses. The irradiations are given at intervals of 4 or 5 days and, as a rule, from 4 to 12 complete the number of treatments. The irradiations are given with a current of 180 kilovolts, 3 ma., a focal distance of 40 cm. and a filter of 5 mm. of lead and 2 mm. of aluminium, through fields of 10 by 15 cm., which receive one-third of the erythema dose, i.e. 250 or 300 r, during each treatment.—C. Guarini, *per J. Amer. med. Ass.*, ii/1937, 749.

Intravenous injection of Frei antigen was found to be the most useful single method of therapy. A uniform routine for the intravenous injection of the antigen is advocated—the patient receiving 0.3 ml. of antigen—the same as that used for testing—on alternate days. Various untoward reactions followed the initial dose, but no alarming reactions were observed in any case.—B. A. Kornblith, *Amer. J. med. Sci.*, 1939, 198, 231.

For the successful treatment of this disease with sulphanilamide, see Vol. I.

Malaria. A group of protozoal diseases caused by different species of *Plasmodium* that invade the red cells of the blood. The group includes:—Quartan malaria caused by *Plasmodium malariae*; benign tertian malaria caused by *P. vivax*; malignant tertian malaria caused by *P. falciparum*; and the malaria due to *P. ovale*. Another form may be due to *P. tenue*, although it is not universally accepted that this is a distinct species. The plasmodium is transmitted by the bite of anopheline mosquitoes, and is characterised by a sexual phase (sporogony) of its life-cycle passed in the mosquito and an asexual phase (schizogony) passed in man. The mosquito becomes infected only by sucking blood containing

gametocytes from an infected human host, and after the sexual phase of the life cycle of the parasite has been completed, the mosquito can transmit infection to another human host. The time taken for the completion of the asexual cycle in man varies with the species of *plasmodium*. For *P. vivax*, *P. ovale*, *P. falciparum* and *P. tenue* it is 48 hours and for *P. malariae* it is 72 hours. The development of fever occurs at the stage of schizogony. Thus in tertian malaria the febrile paroxysms recur every second day and in quartan malaria every third day. (The terms "tertian" and "quartan" denote the recurrence of fever every third and fourth day respectively, counting the last day of the previous attack as the first day of the new.)

In the course of their growth the asexual forms of the parasite cause changes in the red blood cells which are important for the identification of the different species. The most important changes are enlargement, pallor and stippling. Enlargement and pallor occur with *P. vivax*, and to a less extent with *P. ovale*; with *P. falciparum* the red cells are unchanged and with *P. malariae* they are unchanged or darkened in colour; *P. tenue* causes slight enlargement. Two kinds of stippling occur; (1) Schüffner's stippling, in which small red points are evenly distributed throughout the cell, occurs with *P. vivax* and *P. ovale*; (2) Maurer's stippling, in which the points are irregular and less numerous, occurs with *P. falciparum* and *P. tenue*; stippling is not usually seen with *P. malariae*.

Another distinguishing feature is the shape of the gametocytes which develop in the asexual cycle at some stage of the infection. In the case of *P. vivax*, *P. malariae* and *P. ovale* they are rounded or globular, whilst in *P. falciparum* and *P. tenue* they are crescentic.

Diagnosis depends upon finding the malarial parasites in the blood. If they are not found, indications of changes in the blood picture should be sought and the examination repeated later. The patient must not be taking quinine at the time, otherwise the result of blood examination will be negative. To differentiate kala-azar the aldehyde test or the antimony test (see p. 915) should be used.

Diagnosis of Latent Malaria. Technique of new test: A set of 9 dilutions of patients' serum is prepared ranging from 1 : 2 to 1 : 512 in distilled water and to each is added an equal volume (0.4 ml.) of a melanin pigment solution and the series incubated at 37° for 5½ hours. The melanin pigment solution is derived from human hair by hydrolysis with 50% HCl, followed by concentration *in vacuo* and purification by dialysis. Positive results are observed as white granular precipitates forming at the foot of the tube; the degree of positivity is determined by noting the highest dilution of the patients' serum showing precipitation. The rise and fall of the reacting principle in human malarial serum with melanin pigment may thus be quantitatively ascertained during an attack. Reaction shows about the fifth to seventh day after infection, although no parasites may be seen at this stage. The maximum titre of 1 : 128 is reached about the fourth week and then rapidly declines. The authors suggest that the term melanoflocculation test be altered to melano-precipitation reaction.—Greig, Rooyen and Hendry, *Lancet*, i/1934, 1393.

Adrenaline subcutaneously, 0.5 to 1 ml. of a 1 in 1000 solution, cause plasmodia to appear in the blood in 15 to 60 minutes.—per *Prescriber*, 1928, 372

Staining. It is usual to examine both thin and thick blood films spread with a drop of blood taken from the finger or lobe of the ear. Thin films should be spread so that they are a single cell in thickness, allowed to dry rapidly in air, and fixed by flooding them for 10 minutes in absolute alcohol.

Thick films should be spread evenly so that when dry there is no obvious clumping of red cells in the centre.

Muir says the structure of the parasites is well brought out by the following—Soak film in saturated corrosive sublimate solution a few seconds. Wash well, stain with hæmalum 10 minutes, wash, stain again for about the same time with aqueous methylene blue. Wash in water, dehydrate, clear in xylol and mount in balsam. The chromatin of the parasites is violet blue, and the protoplasm pure blue. The Leishman method is, however, principally in use.

Leishman's Stain. Powdered Leishman's stain 0.15 g. in methyl alcohol 100 ml. is good for showing stippling. Stain for 30 seconds, then dilute with about 3 parts of water and continue staining for twenty minutes, wash quickly with water and dry. Instead of diluting with distilled water, Giemsa's stain may be added to the Leishman's stain already on the slide. This gives very intense effects. For both stains, the distilled water used to dilute the stain should be adjusted to pH 7.2.

Gauduchau's Stain. Borrel's blue 6 ml., 1% methylene blue in alcohol (90%) 18 ml., 0.5% water-soluble eosin (blue shade) in dehydrated alcohol 30 ml., dehydrated alcohol 140 ml.

In use, apply the stain, undiluted, to the film, leaving it on about one minute. Then dilute with four parts of distilled water (*neutral*). Each batch of stain requires a different length of time for standing. New stain requires about half an hour, old stain about 20 minutes. In the East, 20 minutes for new stain and 7 minutes for old stain were found sufficient, but in England it takes longer.

Borrel's Blue. Dissolve a small handful of silver nitrate crystals in hot distilled water in a 100 ml. flask. Fill up with 10% caustic potash solution. Wash the resulting precipitate of silver oxide about 12 times in boiling distilled water, then fill up the flask with saturated aqueous solution of medicinal methylene blue. Plug the flask loosely with cotton wool and place in direct sunlight for a day or two (this prevents subsequent precipitation when the stain is exposed to light during later use). Then cork, place in the incubator at 37° for one month, removing cork and shaking occasionally. Filter at end of month; the filtrate = Borrel's blue.

Manson's Method for Demonstrating Flagellate Bodies in Malaria. Blood films are dried and fixed in absolute alcohol (5 minutes). Hæmoglobin is washed out by dropping on 15% acetic acid. The film is then washed in water and stained for 6 hours or longer in 20% carbol fuchsin. It is then washed, dried, and mounted as usual.

Cultivation. Knowles recommends the following method for the cultivation of malaria parasites as an aid in diagnosis. 5 ml. of blood is drawn from a vein with a sterile syringe, the interior of which has been washed with saline. The blood is then added to a sterile stoppered flask containing glass beads, and defibrinated. Sterile stoppered tubes $12\frac{1}{2} \times 1\frac{1}{2}$ cm. are used for the cultures and into each is pipetted a drop of 50% aqueous solution of the *purest* dextrose which has been sterilised by the intermittent method, and to this is added defibrinated blood to a depth of $2\frac{1}{2}$ cm. The upper part of the tube is warmed and a rubber teat immediately fitted to the mouth; this produces partial anaerobiosis. The cultures are incubated at 37° and examined after 12 hours and if necessary after 24 and 48 hours. This is done by aspirating with a capillary pipette some of the upper layer of the sedimented red cells and making films which are stained in the usual way.—Muir and Ritchie, 10th Edn., 1937.

Pink Disease. *Syn.* SWIFT'S DISEASE, FEER'S DISEASE, ERYTHRODEMA, ACRODYNIA. The disease is observed mostly in children, the great majority of the patients being under 4 years of age. Symptoms appear most commonly between 6 and 12 months of age and are as common in breast-fed as in bottle-fed babies, and according to some reports it is more common among well-to-do families than among the poorer classes. It causes much misery, flabbiness and wasting. The onset is insidious, the duration is long, usually six or seven months, the mortality is low, relapses are rare, and sequelæ are unknown.

The course of the disease is divided into three stages, the first two each lasting one month and the third much longer. During the first few days there may be an acute phase consisting of coryza, pyrexia and general malaise. Mental changes become apparent, and there is often lethargy by day and insomnia at night. In this stage also develop tachycardia, progressive muscular weakness, wasting, loss of appetite and increase of thirst, photophobia, intense itching of skin, pains or burning sensations in the extremities (acrodynia), and profuse sweating with sudaminal rashes (pink disease). During the second stage the hands and feet become swollen, red and cold (erythroedema), the palms and soles are sodden with perspiration and desquamate extensively. The outstanding feature of this stage is the intense misery of the child and the pathetic facial expression. Secondary infections through the abraded skin are not uncommon and also in the mouth through sucking of the fingers. Broncho-pneumonia or gastro-enteritis may supervene. The disease has, however, a natural tendency to improve in the third month irrespective of the type of treatment, but convalescence occupies many weeks and the hypotonia is the last symptom to disappear.

Ætiology. The ætiology of the disease is unknown. One view is that it may be an inflammation of the nervous system with an intimate relationship to epidemic encephalitis. Another is that the pathological manifestations may be ascribed to a virus whose toxin is distributed throughout the body rather than acting entirely on the nervous system.

The therapeutic success in isolated cases with vitamin B₁ has led to the theory that the disease is a nutritional deficiency disease, but its occurrence among well-nourished infants is against this view. It has been suggested that in Yugoslavia pink disease is due to ingestion of the spores of a cereal smut (*Ustilago maidis*). Examining this theory in Australia it was considered that the smut *Tilletia tritici* might be an ætiological factor, but experiments on laboratory animals failed to give any confirmation that smut-infected cereals play any part in the causation of the disease.—F. W. Clements, *Med. J. Aust.*, 1940, 2, 430.

Treatment in the open air is of the greatest value and the body should be lightly clad, both by day and night. A tepid bath should be given night and morning and, after drying, the skin of the whole body should be gently rubbed with methylated spirit, followed by a liberal dusting with talc or zinc and starch powder. When the patients are treated in hospital wards, cross-infections, such as broncho-pneumonia and gastro-enteritis, are especially liable to occur and constitute a grave danger to life.—A. J. Wood and I. Wood, *Brit. med. J.*, ii/1935, 527.

Rapid improvement in symptoms occurred within a few days in 4 cases following 600 units of vitamin B₁ *per os* daily; condition cleared up within 2 weeks.—G. Forsyth, *Med. J. Aust.*, 1939, 2, 757.

Good results have been claimed for the injection of vitamin B₁ in massive doses, such as 1000 i.u. once a week. Bellergal (one to three tablets daily for several days) is believed to counteract the effects of the disease on the sympathetic nervous system. Some sedative is essential and a mixture of chloral and bromide is probably the most suitable and should be used freely.—*Price's Textbook of Medicine*, 1941.

Plague. The symptoms of plague in man develop within from 2 to 8 days of infection and consist of fever, headache, giddiness, pains in the back and legs, nausea, vomiting, weakness with staggering gait and great prostration. The eyes are bloodshot, there

is "shouting delirium," and patients frequently have an impulse to get out of bed and wander off, regardless of their condition. In 75% of the cases the lymphatic glands in the groin, armpit and other regions are inflamed, infiltrated and much enlarged, constituting the "buboes," hence the name "bubonic plague" frequently given to the disease. The glands usually suppurate in 7 to 10 days. In the remaining cases the lungs may be primarily attacked ("pneumonic" plague), or a severe blood infection may develop ("septicæmic" plague); in both of these, buboes are absent, or are a late development if the patient lives. Occasionally an eruption of pustules, vesicles or an area of gangrene resembling carbuncles appears on the skin.

The disease is caused by *Pasteurella pestis* which gains entrance to the body through the skin, usually by the bite of a flea, which conveys the infection from rat to man, but it may enter through a cut or abrasion. The pneumonic form is highly infectious owing to the presence of large numbers of the plague bacillus in the expectoration, from which it is readily disseminated in the air.

Morphology. *Pasteurella pestis* is a short fat bacillus, about $1.5\mu \times 0.7\mu$. On staining with weak aniline dye it shows marked polar staining. Spores have not been demonstrated. Non-motile. Does not retain the stain when treated by Gram's method; grows well on usual media both aerobically and anaerobically. The optimum temperature for growth is below 37° —primary cultures grow best at 27° ; minimum temperature 14° . Does not liquefy gelatin. Occurs in chains when grown in fluid media. Forms typical stalactite growths in bouillon with drops of sterile oil on the surface, but must be kept undisturbed. In the tissues and in cultures grown at 37° a typical capsule may be observed and an envelope has been demonstrated by staining with Indian ink. The bacillus produces alkali in its growth equivalent to 1.5 to 2.5% normal sodium hydroxide solution in 6 to 8 weeks. This effects arrest of growth, but not death of the bacillus.

Transmission. The flea, usually *Xenopsylla cheopsis*, is the transmitter from rat to rat and from rat to man. *Rattus rattus* is the species of rodent most likely to give rise to severe epidemics of human plague. Other rodents may assist in the dissemination of the disease and in times of epidemic even domestic animals may suffer from pneumonic plague and become sources of infection. Man may carry plague-infected fleas about his person or belongings and the fleas may infect rats in a new locality. Dissemination may also occur by rats transported with cargoes by sea or rail. Climate plays an important part in the prevalence and periodicity of plague, and epidemics do not occur when the temperature rises above 80°F . with a low humidity.

It is now recognised that certain forms of merchandise, especially grain and to a lesser extent raw cotton, because of the transported rats and fleas, are more to be dreaded as vehicles of plague infection than the human being *per se*. —W.O. Memo. Med. Dis., 1941.

Cultivation. Observations on the plague bacillus show the susceptibility of this organism to atmospheric oxygen. If small numbers of bacilli are inoculated into broth made in the usual way, growth may fail to occur. This appears to be due to a process of oxidation occurring in the broth as the result of exposure to air after sterilisation. If the broth is made in the way recommended in a previous paper by the author (*J. Path. Bact.*, ii/1933, 257), the peptone being added in an early stage of preparation, this process of oxidation does not take place, and the plague bacillus is able to grow satisfactorily. Direct observations show that if

the bacilli are exposed in a thin layer on an agar plate to the action of air, particularly at 37°, they die off fairly rapidly. This can be prevented either by exposing the organisms to an atmosphere with an oxygen content of not more than 1%, or by adding blood, serum, or a reducing agent such as sodium sulphite to the agar. In broth prepared according to the author's formula no destruction occurs, apparently because the oxygen content of the medium at 37° is under 1%. The difficulties of cultivation on solid media may be surmounted by using 0.1% blood agar or 0.1% sodium sulphite agar. Without these additions no growth occurs unless heavy inocula are used. The curious observation was made that, even though in the absence of a protective reducing agent such as blood or sulphite the bacilli are rapidly killed by exposure to air, in the presence of such an agent growth occurs more profusely under aerobic than under anaerobic conditions.—H. D. Wright, *J. Path. Bact.*, ii/1934, 381, per *Brit. med. J.*, i/1935, 34.

Antigenic Structure. *Past. pestis* contains two types of antigens—a heat-stable somatic antigen, and a heat-labile antigen associated with the envelope found in cultures grown at 37°. This latter may be of importance in relation to the immunising properties of a vaccine, for a vaccine made from cultures grown at 37° is said to be of greater immunising value than one made from cultures grown at 25°–30° (as in Haffkine's method).—Mackie and McCartney.

Prophylaxis. This consists in the destruction of rats and fleas and in increasing individual resistance by the injection of Haffkine's prophylactic vaccine.

Haffkine's Plague Prophylactic (Plague Vaccine of the Lister Inst.). Haffkine's vaccine is prepared from a 2 to 6 weeks' culture of *Past. pestis* incubated at 25° to 30° in goat digest broth, killed by heating for one hour at 65°; 0.5% phenol is added to maintain sterility (Topley and Wilson).

After injection there is a local swelling and probably general malaise and heightened temperature. Immunity is conferred after 7 or 8 days by an injection, and it is advisable to inoculate persons exposed to infection every six months. (Dose: 4 ml. for adults or 3 ml. when administered within 3 months of date of manufacture, subcutaneously in any loose tissue free from veins, e.g., the flank.) Over forty million doses of this vaccine have been issued up to 1938.

Living Non-Virulent Vaccine. A new vaccine has been prepared consisting of a living, avirulent organism. The plague strain "Tjividej" accidentally discovered, is so avirulent that both rat and guinea-pig can stand a whole culture. The investigations which have been carried out with strain "Tjividej" show that it is protective in very small doses to those extremely susceptible animals, the house rat and the guinea-pig. Whereas with a dead vaccine in the case of the rat, even after 3 injections, not more than 30% survive, there is a survival of some 85% when inoculated with a single dose of the living vaccine. In the case of man, injections of 1/50 and 1/25 agar culture results in quite a slight and rapidly disappearing reaction. The temperature was raised merely some tenths of a degree. After extended animal trials and trials on volunteers the vaccine was made available for large-scale operations in Java and these were carried out on an alternate case basis. A total of 35,435 persons in two sub-districts were inoculated, the adults with 1/10, the children with 1/25 agar culture, and 44,757 were not inoculated. The respective mortalities were 38 and 213, or 1.01 per thousand as against 4.75. The duration of immunity is a matter of great importance, but there seems little evidence that this extends to more than the usual 6 or 8 months.—L. Otten, per *Trop. Dis. Bull.*, 1936, 365.

(More than a million Javanese were inoculated with this live vaccine without accident and it is claimed that the mortality rate has been reduced to 10% of that among the uninoculated patients.)

A living vaccine made from the EV strain of attenuated *Past. pestis* used on a large scale in Madagascar with about two-thirds reduction in mortality.—G. Girard and J. Robic, *Bull. off. int. Hyg. pub.*, 1936, 28, 1078.

A living vaccine prepared at the S. African Institute for Medical Research from both the EV strain and the "Tjividej" strain which have undergone spontaneous attenuation given to 1000 people without any serious reaction. A dose of 1000 million living organisms is injected, the material having been kept in the ice chest for 5 to 15 days. Five people who had been inoculated from 0 to 6 days after contact with a known case of pneumonic plague all

contracted the disease and died. One patient exposed to infection after inoculation recovered. Of 84 other persons inoculated either within seven days of contact or before contact, none developed plague.—E. Grasset, *Sth. Afr. med. J.*, 1941, 15, 373; also *Brit. med. J.*, 1/1942, 236.

Vaccines made from cultures at 27° are appreciably superior in protective power to those incubated at 37°; heat-killed vaccine is 150 times as potent as the live avirulent vaccine made from the same strain; even guinea-pigs are protected as easily against plague as are mice with heat-killed vaccines; the supernatant fluid in Haffkine's vaccines is protective, while the sediment is not. Virulent plague cultures can be rendered avirulent by sub-culturing them at 37.5° for about 60 to 70 weeks.—S. S. Sokhey, per *Trop. Dis. Bull.*, 1937, 403.

From the practical and administrative point of view immunisation with live plague vaccine (e.g., the "Tjiwidej" strain) has the great advantage of requiring only a single injection instead of two with the killed vaccine, thus allowing mass inoculation to be carried out in a much shorter time. Its employment is simple and without danger and there is little pain and reaction. It is also more economical.—E. Grasset, *Trans. R. Soc. trop. med. Hyg.*, 1942, 35, 203.

Treatment. The only treatment of any value, and then only in the early stages of bubonic plague, is the administration of an anti-plague serum, such as Yersin's. Treatment with the sulphonamides is under trial and preliminary reports are encouraging. Iodine intravenously, in a dose of 7 to 10 m. twice daily of the undiluted tincture, has been widely used in India.

Yersin's Serum (also used as a prophylactic) is supplied in 20 ml. bottles. **Dose:** At the earliest possible moment 50 ml. intravenously and 50 to 100 ml. intramuscularly or subcutaneously, e.g., in the flank, repeated in 12 to 24 hours. 20 ml. is given as a preventive.

Haffkine's Anti-Plague Serum. An anti-plague serum prepared by the Haffkine Institute (Bombay) from cattle (bullocks and buffaloes) using a highly virulent strain of *Past. pestis*, shown far superior to any other anti-plague serum tested. Of a series of 76 cases, 43 treated with the serum showed 14 deaths, while of 33 controls treated without serum, 23 died.—B. P. Naidu and D. P. H. Brist, *Lancet*, ii/1931, 896.

During an epidemic in Bihar 82 patients who served as controls were treated with iodine intravenously; 45 (or 52.4%) died. Of 70 patients given anti-plague serum 20 (or 28.5%) died. Of 53 given sulphapyridine 13 (or 24.5%) died, and of 32 given sulphathiazole only 5 (or 15.6%) died. If patients with septicæmia at the beginning of treatment are considered, 95% of the controls died, as did 60.6% of those given anti-plague serum, while of those given sulphapyridine and sulphathiazole 43.3% and 41.8% died.—Wagle *et al.*, *Indian Med. Gaz.*, 1941, 76, 29.

Pneumonia. *Diplococcus pneumoniae* (*Pneumococcus*) is responsible for more than 90% of cases of lobar pneumonia. Oval or lanceolate, about 1 μ in long diameter, in pairs with the rounded ends together. Has a capsule but this is less evident in cultures. Gram-positive. Grows best in media containing blood or serum. Optimum temperature 37°, does not grow below 25°. Addition of 0.1% glucose to medium favours its growth; often advantageous to incubate in atmosphere containing 5% CO₂.

When grown on blood agar the pneumococcus is non-hæmolytic and the colonies have the same green appearance as the "viridans" type of streptococcus. Under anaerobic cultivation it may be hæmolytic but the hæmolysin is destroyed by oxidation.

Ferments glucose, lactose and saccharose, but *does not ferment inulin*. *Soluble in bile* (1 part of sterile ox bile, or 1 part of 10% solution of sodium taurocholate in normal saline, added to each 10 parts of broth culture; or 0.1 ml. of 10% solution of sodium desoxycholate added to 5 ml. of broth culture not more acid than pH 6.8).

Types of pneumococci. Thirty-two types of pneumococci, differing in their agglutination reactions with type-specific antisera have been recognised, but it is probable that Type XXVI is identical with Type VI and Type XXX is identical with Type XV. For details and description of methods for determination of Types, see Vol. I, p. 1038.

Description of 17 new types in addition to Types 1 to 32.—A. W. Walter *et al.*, *J. Immunol.*, 1941, 41, 279.

Cultivation. Broth used in the preparation of media may be unsuitable for the cultivation of the pneumococcus if it contains copper, or if it is incompletely reduced. Use copper-free peptone and avoid copper vessels and utensils. The peptone should be added to the medium before heating so that it is later subjected to the reducing action of the meat infusion. The method of H. D. Wright is as follows:—

To 1 litre of distilled water add 10 g. of peptone, 3 g. of sodium chloride and 500 g. of finely minced veal, free from excess fat. Mix well and heat for 20 minutes at 68°, stirring at intervals. Shake well, heat in steriliser for 30 minutes, filter through paper, adjust reaction to pH 7·8–8·0. Again steam for 30 minutes and filter through paper. Adjust reaction to pH 7·6–7·8. Add 1·5 g. of glucose. Sterilise in autoclave at 10 lbs. pressure for 10 minutes.—Mackie and McCartney.

The introduction of sulphonamide therapy has made the isolation of organisms from the body fluids of patients under treatment often very difficult; cultures may be negative when viable organisms are present owing to the *in vitro* bacteriostatic effect of the drug present in the inoculum. The difficulty may be overcome by the addition of *p*-aminobenzoic acid to all routine culture media in a concentration of about 5 mg. per 100 ml. during their preparation; it will stand autoclaving at a pressure of 15 pounds for 20 minutes. If sputum from a patient with pneumonia who has been on chemotherapy is emulsified with an equal amount of a 1% solution of *p*-aminobenzoic acid in saline and injected into a mouse, typable pneumococci may be obtained more rapidly and more regularly than when the sputum is injected alone.—C. A. Janeway, *New Engl. J. med.*, 1/1941, 813.

Specific Substance of Pneumococci. In cultures of pneumococci a specific substance is produced by the organism which gives a precipitation reaction with antiserum for the type of pneumococcus concerned. This specific substance is apparently a polysaccharide. Similar but chemically different specific polysaccharides are obtainable from each of the different types of pneumococci. The formation of these substances is closely connected with the virulence and capsulation of the infecting organism. Although the polysaccharides themselves have no toxic action, each is able to destroy the bactericidal effect of whole blood for pneumococci of the type which produced it.

Cultures may show transformation from the typical smooth (S) type to the rough (R) form. This change is accompanied by loss of capsule formation, type specificity and virulence.

Urinary Precipitation Test. The specific polysaccharide substance for Types I, II and III are excreted in the urine in most cases of pneumonia due to these types. They are readily detected by a precipitation reaction between the urine and the type-specific antisera. The test is conducted as follows:—Phosphates are first deposited by adding 4 to 5 drops of N/1 NaOH to 5 ml. of urine in a centrifuge tube and spinning until the supernatant fluid is clear. On top of 0·3 ml. of this urine in each of three Dreyer's agglutination tubes is layered 0·2 ml. of a 1 in 5 dilution of type I antipneumococcal serum (to the first tube), 0·2 ml. of type II antiserum (to the second tube), and 0·2 ml. of type III antiserum (to the third tube). The tubes are immediately examined in a good light for a white ring at the junction of the fluids and re-examined for a deposit after standing on the bench for four hours, the amount of deposit being recorded as +, ++, or +++.

In the examination of over a thousand specimens from 200 patients the test has proved to be absolutely specific. The test is not to be relied upon for early diagnosis but is a useful confirmatory test, especially in type II infections. It may also on occasion identify the infecting pneumococcus type when other methods fail. It is also of value in prognosis, since of 62 patients who on

admission gave a positive urinary precipitation test 24 died, or 38·7%, whereas out of 53 patients with persistently negative tests only 1 died, or 1·9%. It is therefore suggested that in the absence of definite contra-indications a patient with lobar pneumonia whose early urinary specimens contain pneumococcus polysaccharide is a suitable case for serum therapy.—R. Cruickshank and J. A. Montgomery, *Lancet*, i/1938, 217.

Antipneumococcus Serum, Types I and II. Standard, B.P. The standard is a quantity of the dried serum (of either type) prepared and kept at the National Institute for Medical Research, Hampstead.

Unit. The unit is the specific neutralising activity for suitable cultures of *Diplococcus pneumoniae* (Type I or Type II) contained in such an amount of the standard preparation as the Medical Research Council may from time to time indicate as the quantity exactly equivalent to the unit accepted for international use.

Method of Comparison. The following is an abbreviated account of the method given in the B.P. Add. I.

The potency of a sample of antipneumococcus serum (type I or II) is determined by injecting into groups of mice mixtures of graduated quantities of it and of the test dose of a culture prepared from a highly virulent strain of *Diplococcus pneumoniae* and comparing the mortality rates with those produced by injecting at the same time into other groups of mice mixtures of known quantities of the standard preparation and the test dose of the culture. Graduated quantities of the serum being tested and of the standard preparation are chosen, the differences being such that mixtures, containing the larger quantities of serum being tested and of the standard preparation, may be expected to protect all, or nearly all, the mice injected, and that the smaller quantities of the serum being tested and of the standard preparation, may be expected to protect few or none of the mice injected. There are two methods of procedure (a) the method of intraperitoneal injection of mixtures of the serum being tested and the test dose of the culture, and (b) the method of intravenous injection of the serum being tested followed by the intraperitoneal injection of the test dose of the culture.

Friedlander's Pneumobacillus is present in only a small proportion of cases of lobar pneumonia but is common in catarrhal conditions of the respiratory tract, empyema, conjunctivitis, nasal sinusitis, etc. Gram-negative, but stains well with carbol-fuchsin. A bacillus varying considerably in length; usually short with rounded ends. Non-motile, usually $1\mu \times 2.5\mu$. Has a capsule. Is easily cultivated on all ordinary media.

Generally resembles the *B. coli* group in cultural and biological characteristics; some strains correspond closely with *B. aerogenes*, others are non-lactose fermenters. Most strains give negative indole reaction and positive methyl-red reaction (see p. 889).

Three serological types (I, II and III) have been differentiated, and a heterogeneous group which cannot be serologically identified with any of these types forms Group IV. The type-specificity depends upon a carbohydrate contained in the capsule; the specific substances of one of the types is identical serologically with the specific substance of Type II pneumococcus.

Friedlander's bacillus has long enjoyed a reputation for causing a severe form of pneumonia. Bacteriological examination of a large series of pneumonia cases puts the incidence at 0·5 to 1%; occasionally epidemic incidence is reported. Friedlander's pneumonia attacks mostly men in the older age groups. Haemoptysis may be an initial sign; the sputum is characteristically gelatinous and often brick-red; temperature not usually high; the patient becomes quickly ill but physical signs of pneumonia are often absent; there may be leucopenia or slight leucocytosis. A bacteriological examination of the sputum should be made for diagnosis. Gram-stained smears show many typical Gram-negative bacilli with negative halo representing the capsule. Mouse inoculation yields pure culture of Friedlander's bacillus. The infection is usually acute and rapidly fatal (70 to 90%). A more chronic form leading to non-putrid necrosis of the lung, or lung abscess described by S. J. Solomon.—*J. Amer. med. Ass.*, i/1940, 1527.

In an aggregate of 17,260 cases of pneumonia observed by fourteen different authors and their associates Friedlander's bacillus was the causative agent in from 0·6 to 13%. An average of the composite statistics, 196 cases, reveals that roughly 1 in 100 cases was ascribed to this bacillus. The same evidence

shows the infection to be highly fatal, that it usually occurs among people of middle and old age, and is extremely rare in infants and children. Therapeutic reliance should be placed on antiserum since Friedlander's pneumonia is stated to be among the infections in which sulphonamide compounds are of doubtful value.—L. A. Julianelle, per *J. Amer. med. Ass.*, ii/1941, 1466.

Poliomyelitis, Epidemic. *Syn.* ACUTE POLIOMYELITIS, POLIO-ENCEPHALITIS, POLIO-ENCEPHALO-MYELITIS, INFANTILE PARALYSIS.

An acute febrile disease, incident chiefly upon children, though no age is exempt. It is often accompanied by signs of meningeal irritation, followed by nervous symptoms indicative of damage to the central nervous system and occasionally the peripheral nervous system. The lesion of the spinal cord produces acute atrophic paralysis; that of the cerebrum produces somnolence, convulsions and hemiplegia; that of the brain stem causes disturbance of the cranial nerve nuclei; that of the cerebellum, ataxy; and the lesions of the peripheral nerve trunks cause peripheral pains, tenderness of muscles and peripheral facial palsy.

Ætiology. There seems to be general agreement that the causal agent of poliomyelitis is a filter-passing virus which attacks the nervous system, causing inflammation of the grey matter, especially of the anterior cornua of the spinal cord. Occasionally the whole thickness of the spinal cord may become involved. The brain may be primarily or secondarily affected (polio-encephalitis and polio-encephalo-myelitis) and the meninges implicated.

Suggestions have been made, particularly by American workers, that the disease is due to a streptococcus (*vide infra*), but this view is not widely held.

Berkefeld V, N and W and Seitz filtrates of glycerolated tissue taken from monkeys and a mouse during acute attacks, and also a chick-mash culture of the streptococcus from poliomyelitis were examined under a magnification of 12,000 diameters with the electron-microscope. The smallest forms seen resembling organisms approximated in size to $8\mu-17\mu$, the postulated size of the virus particle (E. C. Rosenow, *Proc. Mayo Clin.*, 1942, 17, 99). Though cultures of emulsions in dextrose brain broth of the different strains of what Rosenow calls viruses usually yielded the streptococcus only 4 of 22 filtrates yielded pure growths of the streptococcus in this medium. No organism similar to Rosenow's and suggestive of playing any rôle in the disease has been isolated by other workers using tissue cultures which have been used to cultivate many viruses successfully and provide extremely favourable conditions for the growth of bacteria. The virus of poliomyelitis will survive 6-8 years in 50% glycerol, whereas the streptococcus of Rosenow becomes inactive after little more than a year. Streptococci in any form would be unlikely to survive the treatment with ether which various specimens of fæces, flies and sewage have undergone before being inoculated into monkeys intraperitoneally and intranasally and producing the disease. There is every possibility that the virus of poliomyelitis (estimated size $8\mu-17\mu$) can be seen with the electron-microscope, but any

attempt to relate these bodies to a bacterium and describe them as micrococci is only confusing the issue.

Clinical and pathological evidence in man as well as experimental evidence in monkeys gives no support to the view that the olfactory mucosa is the normal portal of entry of the virus. Experimentally the virus can be transmitted to monkeys by feeding and in man the virus has been isolated from the faeces and in epidemic areas from the sewage. The peak of incidence of poliomyelitis occurs at the same time as that of typhoid. The similarity of poliomyelitis is further illustrated by the isolation of the virus of poliomyelitis from flies in the neighbourhood of infected cases. House flies contaminated artificially will carry the infection for several days and attempts to transmit the experimental disease by the stable-fly *Stomoxys calcitrans* have been successful. The evidence suggests that the gastro-intestinal is the most common portal of entry of the poliomyelitis virus. Efforts to control poliomyelitis as if it were a droplet infection have not been too successful; in the future it would be well to investigate and control each epidemic of the disease as if it were an outbreak of typhoid rather than of influenza.—*Brit. med. J.*, ii/1941, 811.

Epidemiology. Tends to occur in temperate rather than torrid zones; well marked seasonal prevalence in the warm months of the year—July, August and September. In England and Wales 1159 cases of poliomyelitis were reported in 1926 with 176 deaths; in the same year there were 138 reported cases of polio-encephalitis with 59 deaths. The number of cases of poliomyelitis reported in 1933 and 1934 were 714 and 890 respectively.—Sir A. S. MacNalty, *Brit. med. J.*, ii/1936, 59.

Clinically it is observed that cerebral cases usually occur at ages over five years, whereas below this age the spinal form is almost always met with.—*Rep. med. Offr Minist. Hlth, Lond.*, 1936, 42.

The number of cases of acute poliomyelitis during 1938 was 1489 (with 172 deaths) or approximately double the number in 1937; 1938 was the peak year since notification became compulsory in 1912. Unfortunately these numbers do not represent the actual prevalence as there is no doubt that mild and abortive cases which escape detection occur, particularly at times when the reported prevalence is low.—*Rep. med. Offr Minist. Hlth, Lond.*, 1938, 28.

Diagnosis. During the stage of acute pyrexial symptoms and before the nervous manifestations appear, a definite diagnosis can hardly be made; but it may be suggested by the time of year (the summer months), by the prevalence of an epidemic or by the combination of a polymorphonuclear leucocytosis in the blood with a lymphocytosis in the cerebrospinal fluid.

Precipitin and Cutaneous Reactions. By means of a skin test with poliomyelitis antistreptococcus serum it is possible to determine in ten minutes, and by means of a precipitin test within a few hours, the nature of an illness suspected to be the beginning of an attack of acute poliomyelitis. The precipitin test is performed by layering in small tubes, the clear serum of the fasting patient over the serum of horses hyper-immunised to the poliomyelitis streptococcus. Readings are made 90 minutes after incubation at 35° and again after being in the refrigerator overnight. The cutaneous test is made by injecting superficially into the epidermis approximately 0.003 ml. of a 10% saline solution of the wet centrifuged cuglobulin from the serum of horses hyper-immunised to the streptococcus. If the results of the test are positive, an erythematous-œdematous reaction occurs almost immediately which reaches its maximum usually in 10 minutes. The area of erythema is then outlined with pen and ink and duplicated on tissue paper. From the average diameter the area of the reactions is calculated in sq. cm. The œdema immediately surrounding the point of injection is usually directly proportional to the surrounding, more transient, erythema. A greater reaction to the poliomyelitis antistreptococcus serum than to other antistreptococcus serums (encephalitis, arthritis) and normal horse serum (diluted 1 in 10) is considered positive and indicative of an infection by streptococci antigenically related to the streptococcus of poliomyelitis with which the reacting serum was prepared. This reaction to antistreptococcus serums occurs earlier, is not associated with itching and is more erythematous than the reaction which is associated with itching and pseudopodia caused by sensitiveness to horse serum. The therapeutic injection in this early, prepoliomyelitis, infective stage of the poliomyelitis antistreptococcus serum to which the patient's skin and serum react, should result in cure without invasion or

damage to the central nervous system, because recovery without paralysis has occurred often when injections of the poliomyelitis antistreptococcus serum were given in the early stages of active poliomyelitis and in abortive attacks when the spinal fluid was normal.—E. C. Rosenow, *Proc. Mayo Clin.*, 1937, 531.

The cutaneous test investigated in 271 cases of the disease, 150 contacts and 767 controls who gave no reaction to normal horse serum. The reaction was greatest in degree and incidence in the poliomyelitis patients, next among the contacts, then among the controls within epidemic zones and least of all among controls outside epidemic areas. The reaction to serum prepared from streptococci associated with other diseases had about the same incidence, but was much less. From the naso-pharynx of patients who gave a positive reaction to the poliomyelitis serum, a streptococcus was isolated which produced flaccid paralysis in animals. The author considers the test is of diagnostic value in clinical and sub-clinical anterior poliomyelitis, and shows that the streptococcus is as much an active part of the infection as the virus.—E. C. Rosenow, *Proc. Mayo Clin.*, 1939, 734.

Prophylaxis. Active immunisation by vaccine is not considered safe since in some cases the procedure has resulted in the occurrence of the disease in the inoculated person. Passive immunisation by means of convalescent serum is not possible on a large scale because of the limited availability of such serum and the low incidence of the disease in contacts. (The parents' blood is often efficacious when no other serum is available.) Spraying of the nose with zinc sulphate and other solutions has been advocated as a preventive measure (see Vol. II, 21st Edn.), but the results have not been encouraging and it may lead to permanent loss of smell.

Treatment. During the acute stage absolute rest in bed is essential and a light diet. Salicylates relieve the pain and fever and seem to be beneficial, and atropine is of value where the respiratory muscles are seriously involved. In these latter cases artificial respiration is essential to maintain life and the patient should be placed in some form of mechanical respirator such as the Drinker respirator. Convalescent serum has been widely used, but recent evidence indicates that it is of little value. If used, it should be injected as early as possible, from 100 to 200 ml. being given intravenously as soon as the diagnosis is made. (For other references see Vol. II, 21st Edn.)

Directions for the collection and preservation of convalescent poliomyelitis serum.—S. Flexner, *J. Amer. med. Ass.*, i/1928, 24.

Many workers have observed that, left to themselves, patients untreated by serum present paralyses neither more numerous nor more general nor of longer duration than patients treated with serum. At the onset it is impossible to foretell whether or no a patient will develop paralysis, and consequently according to the older conception of the disease whether he is at that time in the preparalytic stage of the disease or in the critical stage of a nonparalytic (abortive) form. It is true that many observers have reported beneficial clinical effects from the use of serum, but statistical proof of its efficacy or otherwise, in the absence of strict control groups, is impossible. The complete absence, however, of any contra-indication or of any phenomena indicating intolerance, may reasonably be held to justify the continued use of serum from suitably selected convalescents.—*Rep. med. Offr Minist. Hlth, Lond.*, 1935, 45.

The value of serum in the treatment and prophylaxis of poliomyelitis is still doubtful.—*Rep. med. Offr Minist. Hlth, Lond.*, 1936, 42.

In the 1937 epidemic in Toronto the incidence of paralysis and the mortality were no less in a group of patients who had 50 ml. of convalescent poliomyelitis serum in the pre-paralytic stage than in those who had no serum. The severity of the paralyses was slightly greater in the untreated cases.—N. Silverthorne *et al.*, *Canad. publ. Hlth J.*, 1941, 32, 410.

Symposium on poliomyelitis.—*J. Amer. Med. Ass.*, ii/1941, 267-282.

Psittacosis. An acute infective disease of parrots and parakeets, due to a filter-passing virus, which may infect human beings.

The disease is characterised in man by an atypical pneumonia, weakness and depression, and signs of a profound infection. Time of incubation about 10 days, the symptoms being headache, fever, anorexia, restlessness, delirium, vomiting, diarrhoea and albuminuria, broncho-pneumonia sometimes supervening. Death

occurs in about one-third of cases. Prior to the 1929-30 epidemic the disease was practically unknown in this country.

The infectious agent of psittacosis is the largest of the filtrable viruses, having a diameter of 275 m μ . Of the common laboratory animals, mice, guinea-pigs, rabbits and rhesus monkeys are susceptible to infection with the virus, the mouse being the most susceptible. Mice dying after intra-abdominal inoculation of the virus have multiple lesions of focal necrosis in the liver which may be visible on gross inspection. The spleen is frequently enlarged and may show microscopic evidence of infiltration with mononuclear cells and regions of focal necrosis. The cytoplasm of certain mononuclear cells of the liver and spleen contains masses of virus particles. These apparently represent intracellular colonies of the virus and stain well with Giemsa stain. Impression preparations of the spleens of infected mice usually show such bodies in great numbers. They have a diagnostic significance comparable to the Negri bodies in a case of rabies. As there is considerable variation in the elimination of virus in the sputum of human beings with psittacosis, it is important to make inoculations with several specimens of sputum taken at intervals of 24 hours.—F. R. Heilman, *Proc. Mayo Clin.*, 1940, 662.

Transmission. It is a rather common disease of birds and may be transmitted not only by parrots, parakeets and "love birds," but also by canaries, finches and other species. Close contact with infected birds is not necessary, since aerial transmission of the disease for some distance is known, and dust containing faecal material from the bird's cage may transmit the virus. Epidemics are usually traced to newly-acquired birds; those which have been in a household for a long time are less dangerous. Children are relatively immune and the mortality rate among middle-aged persons is estimated at about 20%. A description of two cases (in one of which sulphapyridine was employed with success).—H. C. Hinshaw, *Proc. Mayo Clin.*, 1940, 657.

History of an outbreak at the London Zoo in February 1938, with an account of a fatal case.—A. G. Troup *et al.*, *Brit. med. J.*, i/1939, 51.

Staining. Psittacosis virus is present in the tissues of infected hosts (especially liver, spleen and lungs) and may be demonstrated by staining with Giemsa's stain or by Castaneda's method of staining as for rickettsiae (p. 940). In smear preparations stained by Castaneda's method, round or slightly oval elementary bodies, 0.2 to 0.8 μ in size, of blue colour, are seen against the pink colour of the cells; these elementary bodies are either lying free or within reticuloendothelial cells.

Complement-fixation Tests are made with an antigen of infected mouse spleen emulsion, or of cultures of the virus in Rivers' tissue culture medium or in the chorio-allantoic membrane of the developing chick.

To prepare the mouse spleen antigen, a 5% emulsion of the spleen from an infected mouse is made in buffered saline and allowed to sediment overnight. The supernatant fluid is removed and centrifuged at high speed. The sediment, which contains the virus, is resuspended in buffered normal saline and heated in a steamer for 20 minutes. This boiled fluid or "cocto-antigen" is used in the complement-fixation test against the patient's serum, using controls against normal sera and known positive psittacosis sera.

The complement-fixation reaction gives valuable assistance in the diagnosis of psittacosis in man. The reaction has been found positive as early as the twelfth day of disease and as late as the fifth week. It must be emphasized that the specific complement-fixing power of human psittacosis sera is of a low order and that in order to detect it a finely balanced test is essential. For this reason rigorous control of the test is absolutely necessary if erroneous results are to be avoided.—S. P. Bedson, *Lancet*, ii/1935, 1277.

Observations on the complement-fixation test in psittacosis, employing a heated psittacosis antigen.—S. P. Bedson, *Lancet*, ii/1937, 1477.

Laboratory diagnosis of psittacosis.—*Rep. publ. Hlth med. Sulj.*, Lond., No. 80, 1937.

The Meyer-Eddie complement fixation test for psittacosis.—*J. Infect. Dis.*, 1939, 65, 225.

Complement fixation test using cocto-antigens from virulent mouse spleens on Rivers-Li tissue cultures of the virus gave positive reactions in human psittacosis as early as the 6th day after infection, and may remain positive for months after the illness—probably indicating that the patient is a virus carrier. Convalescent serum, given intramuscularly and intravenously, was found

effective against psittacosis provided it contained a large number of neutralising antibodies.—K. F. Meyer and B. Eddie, *J. Infect. Dis.*, 1939, 65, 225.

An atypical case in a man aged 69 with clinical features which strongly suggested typhoid fever was diagnosed by a positive complement-fixation test. The unusual features were: periodic occurrence of diarrhoea with "pea-soup" stools; duration of 5 weeks (usual average is 3 weeks); onset by acute illness for a few days, followed by an ambulatory period before onset of characteristic symptoms; absence of headache. There was a typical lung lesion resembling lobar pneumonia, but without sputum, respiratory distress or cyanosis.—R. B. MacMillan, *Brit. med. J.*, i/1940, 613.

Mouse-inoculation Tests may be used for diagnosis during the acute stage of the infection. The sputum is emulsified in saline and centrifuged. 0.5 ml. of the supernatant fluid is inoculated intraperitoneally into each of 4 or 6 mice. In a positive case, some or all of the mice will have died in 7 to 10 days, showing enlargement of spleen and liver and glairy peritoneal exudate. Microscopical examination of smears from the exudate and spleen shows the typical elementary bodies (*vide supra*). If none of the mice die within 10 days, they are killed and an emulsion of spleen inoculated into other mice; if none of these die within 10 days the case is presumed negative.

Rabies. Rabies is an acute infectious disease communicated to man by the bites of infected animals, usually dogs. The disease is due to a filtrable virus which is located in the salivary glands and the central nervous system. The incubation period in man is usually from one to two months, bites on the face, head and neck having a shorter incubation period than those on other parts of the body. The onset is generally sudden, though prodromal symptoms such as fever, headache, depression, restlessness and insomnia may be noted a day or two before hydrophobia sets in. Once clinical manifestations occur the disease invariably ends fatally. In England the disease has been eradicated by the strict quarantine of all imported dogs and no case in man has been reported in this country for nearly 40 years.

Diagnosis. The symptoms and death of a rabid dog are important points in diagnosis and the suspected dog should be chained up, muzzled, and kept under observation; if the dog is still alive at the end of 10 days it is proof that the bitten person has not been infected, since an infected dog never survives longer than 6 days from the onset of illness and the saliva is never infective for more than 4 days before the onset of symptoms.

When the animal dies the head is cut off and the brain removed. Sections and smears should be made of the hippocampus, the cortical grey matter of the cerebrum and the cerebellum. These should be examined for the presence of Negri bodies. These are round or oval bodies from 0.5 to 25 μ in diameter, which are present within the cytoplasm of the cerebral cells and are demonstrable in 97% of dogs infected with street virus. Fixing the tissue in formalin and staining paraffin sections by Leishman's method is satisfactory. Smears may be fixed in methyl alcohol and stained by Giemsa's stain.

Treatment. Bleeding should be encouraged and thorough cauterisation of the wound carried out with strong nitric acid as soon as possible. The wound should not be sutured for three days. Antirabic vaccination should be instituted immediately, either by the inoculation of attenuated, living, fixed virus, as in the Pasteur method, or by the use of carbolised or etherised vaccines in which the fixed virus has been killed, as in the Semple method.

Of the 118,000 persons who received anti-rabies treatment in the different Pasteur Institutes only 408 (0.35%) died. Most of the cases occurred in the east, especially India. In Europe, 8248 cases were treated in Yugoslavia, 1776 in Athens, 1740 in Istanbul, 2844 at Budapest and more than 2000 at Warsaw. The highest mortality, 11.2%, was caused by rabid wolves, as compared with 0.33% caused by mad dogs and 0.03% by cats.—*Brit. med. J.*, ii/1937, 926.

In addition to the original vaccine of Pasteur, in which the virus in the spina cord of infected rabbits is attenuated by varying periods of drying, we now employ vaccines in which other agents, such as phenol, formol, ether, glycerol.

or heat, are used to inactivate the virus. Of these vaccines the carbolised is the most in vogue, whilst vaccines made from dried cord, or cord inactivated by heat or ether or prepared by simple dilution are the next most popular; the other types are but little used. The figures published by the League of Nations for 1929-35 show that only 0.4% of 524,258 people receiving anti-rabies treatment died from rabies.—S. P. Bedson, *Lancet*, ii/1940, 577.

Relapsing Fever. Relapsing fever occurs in many parts of the world and is often seen in epidemic form during wars and famines (hence the old name "famine fever"). It is an acute infectious disease due to spirochaetes transmitted by lice or ticks, the infection being transmitted by crushing the insect on the skin. After a period of incubation of from 5 to 7 days the disease sets in with chill, fever, and pains in the back and limbs. Anorexia, nausea and vomiting are common. The spleen enlarges, sweats and delirium occur, and the symptoms continue for 5 or 6 days, then suddenly cease by crisis. After a variable interval, usually about a week, a second paroxysm occurs, which may be followed by a third and fourth. The prognosis varies; with the European and American types the mortality rate is only 3 to 5%, but with the Asiatic type it is much higher. Jaundice is an unfavourable development.

Aetiology. The different varieties of the disease are caused by spirochaetes demonstrable in the peripheral blood during the febrile paroxysms, and include (1) European relapsing fever, due to *Borrelia* (or *Spironema*) *obermeiri* (or *recurrentis*), (2) Indian or Asiatic relapsing fever due to *Borrelia* (or *Spironema*) *carteri*, and (3) North American relapsing fever, due to *Borrelia* (or *Spironema*) *novyi*. (West African relapsing fever, or African tick fever, is due to *Borr. duttoni*, transmitted by a tick; see Tick Fever, p. 964)

Transmission. The mode of inoculation of the organism from infected lice is by its introduction into abrasions produced by scratching, the crushed insect providing the inoculum. Lice only infect after they have been crushed and the spirochaetes liberated from the coelomic fluid. The spirochaetes can be observed in the stomach of the louse for a day after infective blood has been ingested; after about 6 days they can be demonstrated in the body cavity, and then spread through the insect's body.

Staining. The spirochaetes of all the forms of relapsing fever are alike in their microscopical characters. They occur in the blood as delicate spiral filaments with a length of from 10 to 20 μ and a thickness of 0.3 to 0.5 μ , and show several fairly regular coils about 2 to 3 μ in length and varying in number; their extremities are pointed. They are actively motile and have a peculiar movement partly rotatory and partly undulatory. The most satisfactory method of demonstrating the organisms is by dark-ground illumination; very rarely strains are met with which show double contour. They stain with watery solutions of the basic aniline dyes, though somewhat faintly. They are best coloured by the Romanowsky stain or one of its modifications and then usually have a uniform appearance throughout or may be slightly granular in places. They are gram-negative.—Muir and Ritchie, 10th Edn., 1937.

Cultivation. Kligler and Robertson (1922) found the following medium successful for the growth of relapsing fever spirochaetes:—Horse or rabbit serum is diluted with 1 or 2 parts of saline solution, or undiluted ascitic fluid; to each 10 ml. of this fluid is added 1 ml. of 10% peptone broth and the reaction adjusted to pH 7.2. 3 to 4 ml. of the mixture is placed in each test-tube. Inoculate with a drop of blood or 0.1 ml. of fluid from a previous culture, and cover the surface with a layer of oil. Aristowsky and Holtzer (1924) used a medium prepared by adding 8 ml. of saline solution to 4 ml. of young horse's serum in a test-tube and introducing a piece of blood-clot or white of hard-boiled egg; inoculate and incubate at 35°; subculture every 48 to 72 hours.—Wenyon, p. 1257.

Galloway's method is simple. It consists in placing a small amount of egg-white in a test-tube which is kept slanting in a hot-water bath, so that the

coagulum may have as large a surface as possible, then 5 ml. of a dilution of serum which has been previously heated for one hour at 58° to 60° is added (rabbit's serum is diluted 1 : 5, horse serum 1 : 10) and the mixture is covered with sterile melted soft paraffin. Sterility is controlled by keeping the tube at 37° for 24 hours before inoculation. The addition of a drop of fresh human or rabbit blood favours growth.—Muir and Ritchie, 10th Edn., 1937.

Treatment. Neocarsphenamine in doses of 0.45 to 0.6 g. gives excellent results in relapsing fever. As a routine 7 to 8 doses are necessary, one being given every week, and cases treated at the commencement of the illness respond much more quickly than patients who have had relapses. 1.5 g. of acetarsol given by the mouth on two successive days is said to produce disappearance of the spirochaetes from the blood in 6 to 36 hours and to prevent relapses. The drug has also been used prophylactically.—R. N. Chopra, *Handbook of Tropical Therapeutics*, 1936.

Rickettsia Diseases. The term *Rickettsia* is applied to certain small, non-motile, Gram-negative bodies, of which *Rickettsia prowazeki*, the organism of epidemic typhus, is the type species. These bodies are found to develop in the alimentary tract of certain blood-sucking arthropods such as lice, fleas, ticks and mites, rodents playing an important rôle as reservoirs of infection. Ricketts, in 1909, first noted these bodies in guinea-pigs and monkeys with Rocky Mountain spotted fever, and since then several varieties affecting man have been described, and an ever-increasing number of typhus-like fevers due to rickettsias are being reported from different parts of the world. These have been classified on a geographical basis according to their insect vectors and the table given on p. 939 which was recently issued by the Army Pathology Laboratory Service presents a useful summary of present knowledge.

Epidemic Typhus. *Syn.* TYPHUS FEVER; TYPHUS EXANTHEMATICUS; CAMP FEVER; JAIL FEVER; FAMINE FEVER. A highly contagious fever characterised by rapid onset, severe prostration, a mottled rash and termination by sudden crisis about the fourteenth day. The incubation period is usually from 8 to 14 days (extremes, 4 to 20 days). Though the onset is sudden, it is often preceded by malaise and a rise of temperature. Common initial symptoms are severe headache, giddiness, shivering or rigor and pains in the back and limbs. The face is flushed and the features swollen, the conjunctivæ are injected and there is slight contraction of the pupils. The period of onset lasts about two days and on the third day the symptoms become aggravated, the pulse rate increasing and the temperature rising to 103°–104°F. Marked delirium may be present early in the disease and is usually present after the first week. The rash generally appears on the fifth day, being most profuse on the trunk but often covering the whole body except the face. As the rash develops the headache becomes more intense and the patient becomes dull and lethargic. If he is going to recover the condition suddenly improves on about the fourteenth day. The severity of the disease varies greatly with the age of the patient. From extreme childhood the death-rate progressively rises until, after the age of 50, the disease is practically always fatal.

RICKETTSIAL INFECTIONS IN MAN

| Disease | Rickettsia | Geographical distribution | Insect vectors | Possible vertebrate reservoirs |
|------------------------------|---|---|---|--------------------------------------|
| Exanthematic typhus | <i>Rickettsia prowazeki</i> | Europe, Abyssinia, North Africa, Belgian Congo, Asia Minor, Persia, North China, Mexico | Louse, <i>Pediculus humanus</i> | Man |
| Endemic or murine typhus | <i>R. prowazeki</i> var. <i>mooseri</i> (= <i>R. muricola</i>) | World-wide .. | Rat flea, <i>Xenopsylla cheopsis</i> | Rat (squirrel, shrew) |
| Tsutsugamushi disease | <i>R. orientalis</i> (= <i>R. tsutsugamushi</i>) | Japan, Formosa, Malaya, Java, Sumatra, New Guinea | Larva of <i>Trombicula akamushi</i> (Japan), <i>T. deliensis</i> (Malaya), <i>T. deliensis</i> (India), <i>T. minor</i> (New Guinea) | Bandicoot |
| Trench fever | <i>R. quintana</i> (= <i>R. volhynica</i> and probably <i>R. weigli</i>) | North Africa .. | Louse, <i>P. humanus</i> | Man |
| Rocky Mountain spotted fever | <i>Dermacentor xenus rickettsi</i> | U.S.A. | <i>Dermacentor andersoni</i> , <i>D. variabilis</i> | Goats, hares and other small rodents |
| Fièvre boutonneuse | <i>D. rickettsi</i> var. <i>conori</i> | Mediterranean zone | Dog tick, <i>Rhipicephalus sanguineus</i> | Dog |
| South African tick typhus | <i>D. rickettsi</i> var. <i>pijperi</i> | South Africa .. | Tick, <i>Hemaphysalis leachi</i> | Dog (?) |
| Sao Paulo rural typhus | <i>D. rickettsi</i> var. <i>brasilensis</i> | Southern Brazil | Tick, <i>Amblyomma cajennense</i> | Opossum |
| Q fever .. | <i>Rickettsia burneti</i> (= <i>R. diaporica</i>) | Australia, U.S.A. | Ticks, <i>Hemaphysalis humerosa</i> , <i>Dermacentor andersoni</i> , <i>D. occidentalis</i> , <i>Amblyomma americanum</i> , <i>Rhipicephalus sanguineus</i> (?) | Bandicoot |

The disease is associated with famine and overcrowding, and of the two, the former is the more important. A third factor which is perhaps of even greater importance in the spread of the disease is the widespread movement of military or civilian populations, bringing non-immunes into a district where the disease is endemic or carrying the disease into a typhus-free region. Louse-borne typhus is a disease of cold countries and is unknown in tropical regions though it may occur in the mountainous sectors. Personnel working among typhus patients are exposed to great danger and in view of the lower mortality at early ages only young personnel should be employed.

Bacteriology. The virus now generally considered responsible for the disease is *Rickettsia prowazeki*, first described by da Roche-Lima in 1916. The organisms stain a reddish-purple with Giemsa and are Gram-negative; they are very small, of short elliptic or olive shape, often in pairs, and are surrounded by a paler staining substance; they are pleomorphic; occasional very short and very long forms—up to 1.5 to 2 μ —may be seen. They do not grow on ordinary media, but can be cultivated in tissue cultures and in living chick embryo. Lice which have fed on the blood of infected persons are not able to transmit the disease by their bites for 8 to 10 days, but they then remain infective for the rest of their lives. The virus of typhus fever is present in the blood, which is infective from the onset and continues so until the day after the temperature becomes normal, although the virus is believed to persist in the blood long after recovery from the disease.

Staining. **Castaneda's Method.** For staining *Rickettsie* and elementary bodies, e.g., those of psittacosis.

Monopotassium dihydrogen phosphate 1 g. in distilled water 100 ml.; 25 g. of disodium hydrogen phosphate in 900 ml. of distilled water. Mix the two solutions so that the pH is 7.5. Add 1 ml. of formaldehyde solution as preservative. 20 ml. of this solution are mixed with 1 ml. of formaldehyde solution and 0.15 ml. of a 1% solution of methylene blue in methyl alcohol. This mixture is applied to the film to be stained for 3 minutes and then poured off. Do not wash the film, but counter-stain for 1 or 2 seconds with Safranin "O" 0.2% solution in water, 1 part, and acetic acid, 0.1% solution in water, 3 parts. Wash off in running water, blot and dry. The rickettsie (and elementary bodies) stain blue, the protoplasm and nuclei of the cells are red.

Diagnosis. As an aid to diagnosis the production of artificial stasis of the vessels is said to be useful. Where the rash is not characteristic or is sparingly developed, place a bandage round the arm. The resulting engorgement of vessels shows up the exanthem more clearly and the red maculæ can be observed to take on a blue, cyanotic hue which eventually changes to a brown or coppery colour.—*W.O. Memo. Med. Dis.*, 1941.

Weil-Felix Reaction. The organism isolated by Weil and Felix (designated *Proteus* X19) is so constantly agglutinated in high dilutions by the serum from typhus fever patients, while it is not affected by serum from other conditions, that the reaction possesses great value in diagnosis. The agglutinins for this organism appear in the blood on the fourth or fifth day of the disease and reach their height in the second week. The test should be carried out by the naked-eye method, with living suspensions of agar-slope cultures, the mixtures of serum and organisms being incubated at 37° for two hours. Agglutination with a 1:200 dilution of serum may be taken as a positive reaction. Positive reactions at lower titres should not be regarded as diagnostic unless the titre of the reaction has risen greatly from the onset. Felix recommends the use of a culture of the O variant for the performance of the test, since the agglutinins in typhus fever are of the O type.

The proteus group of organisms grows in two distinct forms: (1) in a continuous surface spreading film of motile flagellate bacilli, the H variety, and (2) in the O form, consisting of separate organisms. Only the O strains of *Proteus* X19 are used, since they contain only the somatic or O antigens, and the agglutinins in typhus fever are also of the O type. There are also two strains, X2 and X19 giving similar reactions, only the latter furnishes them in tubes of 1 in 5000 to 1 in 10,000, which is ten to twenty times the dilution that X2 does

so it is mainly used. A third variety, XX, or the Kingsbury strain, has been found to give reactions in the scrub or country type of Malaya typhus, which gave negative reactions with the X19 type. Both forms are therefore required in carrying out the Weil-Felix reaction in endemic or tropical typhus, the O forms always being used.

A positive reaction at 1:25 during the first week of the disease may be considered as a presumptive positive, though about 7 to 9% of persons not suffering from typhus may react at this titre. As a rule the titre rises rapidly so that a positive diagnosis, 1:50 or higher, can generally be made by the 8th day. The reaction has been employed on a large scale and has been found remarkably valuable.—Topley and Wilson.

The reaction must be interpreted with caution. Conclusive in very high titres, above 1:1000; titres of 1:100 very suggestive, but 1:50 only indicates possibility of typhus. Unusual for reaction to be so pronounced in diseases other than typhus as to be regarded as positive, but it is occasionally positive in scarlet fever and small-pox, and most likely to be so in paratyphoid B fever. Rise of titre during disease a strong argument in favour of typhus.

Rapid Bedside Diagnostic Test. The test is performed with whole blood taken from the ear or the finger. The amount of blood is measured with a platinum loop of 5 mm. diameter and one loopful is mixed with one standard drop of the antigen on a clean slide. The slide is kept moving to and fro during one minute, then the test is read. When the blood and antigen remain in a uniform mixture the test is negative. A positive test shows clumps of the antigen which usually gather round the edge of the drop, forming a ring which can be detected by its whitish colour in contrast with the red cells. No doubtful tests are considered and no readings are reliable after one minute. The antigen is a suspension of *Proteus OX19* in 11:1000 solution of sodium citrate with 0.2% formalin. A 24-hour growth in petri dish is washed in 5 ml. of diluent to obtain the optimum concentration. This emulsion is kept in the refrigerator and gives good reaction for two months. The method is simple and so far no false positive reactions have been observed. It is only qualitative and is not more sensitive than the ordinary tube agglutination.

The antigen is prepared as follows: The bacilli are suspended in physiological saline containing 10% formalin and this emulsion is maintained at room temperature for 72 hours. It is then filtered through cotton and the filtrate is centrifuged, the sediment being resuspended in as small a quantity of 1.1% sodium citrate solution as will produce a suspension. The suspension is standardised and used in various dilutions together with serum of low titres, 1 in 100 and 1 in 1000. The solution which gives rapid and clear reaction with high titre serum, and light reaction with low titre serum should be used, and to make the reaction more clear, enough aqueous methylene blue solution is added to the suspension to give an intense blue colour.—M. R. Castaneda and R. Silva, *per Trop. dis. Bull.*, 1941, 448.

Prophylaxis. Every effort should be made to get rid of lice and nits. The patient should be stripped, the hair clipped short and the body shaved and thoroughly washed before admission to a ward. After the patient's body has been cleansed it is advisable to rub him all over with 10% camphorated oil or to spray him or rub him down with kerosene, benzene or petrol; he should then be isolated for four weeks. Immediate contacts should be isolated for 14 days. Those in attendance on the sick should have their hair cut short and should wear protective clothing (as described in *Ministry of Health Memo.*, 252/*Med.*). Administrative measures of control include the drawing of a cordon round healthy areas, the complete disinfestation of all infected refugees entering that area, and the improvement of the nutritional conditions existing among the population. For the disinfestation of clothes, furniture and premises cyanide gas is the most satisfactory; alternatively, clothing or bedding may be disinfested by the steam process or by soaking in 2% cresol solution for an hour.

For immunisation, vaccines have been used with considerable success. Of these, the best known are Weigl's vaccine made from killed virus obtained from infected lice, Blanc's living virus obtained from murine typhus and Cox's vaccine prepared from rickettsias grown in developing chick-embryo (*vide infra*). In outbreaks of epidemic typhus the live virus is used, but for individual protection in endemic areas the killed virus is safer.

Immunisation Against Rickettsia Infections. A vaccine to be of value against a human rickettsia infection must fulfil certain conditions. (1) It must produce a high degree of immunity, lasting for a considerable time.

(2) It must not produce dangerous reactions, nor render the individual a danger to his companions. (3) It must be easily produced in large quantities and must be easily administered.

Killed Exanthematic Typhus Vaccine. The earliest, and up to the present time, the most widely-used killed rickettsia vaccine is that of Weigl. The method consists of infecting lice per rectum with living *R. prowazeki*. After a suitable time the lice are killed; the intestines swarming with rickettsias are removed and ground up in a dilute solution of formaldehyde in saline. The disadvantages of this method are that a large number of lice (usually between 200 and 300 for the immunisation of a single individual) are required; a large and skilled staff are necessary to superintend the inoculation and feeding of the lice; the cost of inoculation is high; the method cannot be used in countries where lice are already heavily infected with rickettsias of the Rocha-Lima type. A similar method, using ticks instead of lice, has been used for vaccinating against Rocky Mountain spotted fever (see p. 947).

Numerous attempts have been made to overcome the difficulty of obtaining dead rickettsias in sufficiently large numbers. The rickettsias have been grown in serum-tyrode mixtures containing chick-embryo tissues; the resulting growth cannot always be maintained.—(C. Nigg and K. Landsteiner, *J. exp. Med.*, 1932, 55, 653.) Agar slopes on the surface of which was smeared chick-embryo tissue mixed with a suspension of rickettsias were used by H. Zinsser *et al.*, *Proc. Soc. exp. Biol.*, N.Y., 1937, 37, 604.

Injection of rickettsias from typhus, Rocky Mountain spotted fever, fièvre boutonneuse and Q fever into yolk-sac of developing chick-embryo yields a very rich growth of rickettsias. Formalised or phenolised suspensions of rickettsias obtained by this method were used in a Spanish outbreak of typhus. One dozen eggs yield about 100 ml. of vaccine—enough for 30 people.—H. R. Cox, *Amer. J. trop. Med.*, 1940, 463.

The method of P. Durand and P. Giroud (*Arch. Inst. Past., Tunis*, 1940, 29, 25 and 234) consists of setting up a rickettsial pneumonia in mice or rabbits, removing the lungs at the height of the infection and by differential centrifugation of an emulsion, obtaining a rich suspension of rickettsias relatively free from foreign matter. The rickettsial suspension is treated with formalin or phenol. No reactions are caused by the injections of this vaccine, but 4 or 5 injections at intervals of 5 days are necessary. A good immunity against exanthematic typhus is produced, but only partial immunity is produced against murine typhus. Where both exanthematic and murine typhus exist it would seem preferable to employ a vaccine containing a mixture of both types of rickettsias.

No large-scale figures are available to determine which of these various methods of immunisation is likely to be most effective in controlling epidemic exanthematic typhus. Preliminary results with the original egg-yolk vaccine have been disappointing. The use of developing chick-embryo and the fact that a variety of rickettsia can be adapted to the mouse lung justify the hope that the problem of obtaining rickettsias in quantities sufficiently large to constitute efficient vaccines is in a fair way of being solved.—G. W. M. Findlay, *Lancet*, i/1942, 483.

The typhus fever vaccine used by the U.S. Army is a suspension of killed *Rickettsia prowazeki* grown in the yolk-sac of developing hens' eggs. Three injections of 1 ml. each are administered subcutaneously at intervals of 7 to 10 days. Further doses of 1 ml. each are given every four to six months when serious danger of infection exists.—*War Med.*, 1942, 139.

No death from typhus has ever been reported in a patient who had been fully immunised by Weigl's method, but other methods are not so reliable.—J. Mrugowsky, *per Trop. Dis. Bull.*, 1942, 541.

Weigl's vaccine is the only one that has been proved successful on a large scale in human beings. Cox's yolk-sac vaccine is equally effective in producing immunity in animals, but final judgment must await large-scale human experiments.—H. Hetsch, *per Trop. Dis. Bull.*, 1942, 548.

Report of animal protection experiments with vaccine made from emulsion of living tissue of mice infected with rickettsias obtained by rat-guinea-pig passage of L strain virus. For details of practical method for the preparation of large quantities of typhus vaccine, see M. R. Castaneda, *Brit. J. exp. Path.*, 1941, 22, 167.

Living Murine Typhus Vaccine. The rickettsia of murine typhus, *q.r.*, is closely related to that of exanthematic typhus, but produces a much milder

disease; experiments have therefore been made on the effect of injecting murine typhus rickettsias into man. A strain of murine typhus passaged in the brains of mice was employed in Tunis by Laigret (1937-39). The mouse brains are ground up and dried in egg-yolk with sodium phosphate. Severe criticism has been directed against the use of this vaccine. In Europeans, as well as in some natives, severe reactions have followed the injection and the rickettsias of murine typhus have been isolated from the blood; in view of the possible conversion of murine into exanthematic typhus the widespread dissemination of murine typhus among a population is not without dangers. In Spain the Laigret vaccine gave unsatisfactory results.

In Morocco, Blanc and Baltazard used two methods. The first employed infected tissues of guinea-pigs, ground up in physiological saline and treated with bile immediately before injection. The second used the dried faeces of rat-fleas (*Xenopsylla cheopis*) infected with murine typhus rickettsias; the faeces were mixed with bile before injection. In Europeans the reactions from the use of these bile-treated rickettsias are too severe to allow of their general use, and in the case of African natives judgment must be suspended until adequately controlled experiments have been performed.—G. M. Findlay, *Lancet*, i/1942, 483.

The new method of Blanc (biliated flea virus) and that of Laigret-Durand (mouse-brain) are the only ones that can be considered for the preparation of vaccine intended for Europeans, since their products can be titrated and are less likely to induce serious vaccinal reactions. Both are capable of furnishing the quantities necessary for mass vaccination and both have given equally favourable results in suppressing epidemics in N. Africa.—Y. Biraud, *Bull. Hlth Org., L.O.N.*, 1943, 1-64.

Treatment. There is no specific treatment, and treatment should be on the same lines as those adopted in typhoid fever. Complete rest and careful nursing, good ventilation, and a light nourishing diet with plenty of fluids are important. Morphine is a valuable drug and lumbar puncture is distinctly beneficial. Convalescent serum, in doses of 20 to 50 ml., is said to lessen the severity of the symptoms, though having no effect on the duration of the illness.

An anti-typhus serum has been introduced by Nicolle and the reports on its use are distinctly favourable. The daily dose is 10 to 20 ml. hypodermically. Other new methods which have been favourably reported on but require further investigation are autoserotherapy, the serum of a patient's blood taken on the 8th day of the disease and allowed to clot naturally and the serum injected intravenously in doses of 5 ml.; the transfusion of 20 ml. of whole, citrated blood; the injection of the patient's cerebrospinal fluid intravenously or subcutaneously; and the intrathecal injection of 20 to 25 ml. of convalescent serum. (See also *W.O. Memo. Med. Dis.*, 1941.)

The use of pooled convalescent sera was tried in Poland in 1940 but proved ineffective.—R. Wohlrab, per *Trop. Dis. Bull.*, 1942, 547.

Brill's Disease. This disease, occurring among immigrants in the United States, was at one time considered to be a form of flea-borne murine typhus but has since been proved by Zinsser to be a mild recrudescence of louse-borne typhus contracted in childhood in Europe. The disease is not transmitted by lice, so it may be considered non-infectious. It may be differentiated from murine typhus by inoculation of rats which are very susceptible to flea typhus but resistant to the virus of Brill's disease.

Endemic Typhus. *Syn.* MURINE TYPHUS, FLEA TYPHUS, URBAN TROPICAL TYPHUS, HONE'S DISEASE, SHIP TYPHUS, X19 TROPICAL TYPHUS. A mild typhus-like fever, generally occurring in non-epidemic form. It has a world-wide distribution, cases having been reported from seaports in countries bordering the Mediterranean and from South Africa, Malaya, the Dutch East Indies, Manchuria, Cochin-China, the Eastern States of America, Mexico and a number of other countries. The disease is primarily one of rats and mice and is caused by *R. mooseri*, which is conveyed from rat to rat and from rat to man by fleas, the infection being introduced by scratching or rubbing the faeces of the flea

into the skin and not directly by the bite. It has been held that infection may be conveyed to man by direct contact with rats or by eating food contaminated with the urine of infected rats. It is a disease of summer and autumn, when rat fleas are most numerous, as opposed to epidemic typhus which is a winter disease coinciding with prevalence of the body louse. It is much less fatal than epidemic typhus, the mortality being under 5% as compared with 25% or over.

Diagnosis. The mild character of the fever, together with a history of contact with rats known to be infected with the disease, and the strongly positive Weil-Felix reaction to *Proteus* X19 and a negative response to XK, are sufficient to enable a diagnosis to be made.

Complement-Fixation Reaction. A series of clinical experiments was carried out with the object of finding whether the complement-fixation test would be a satisfactory means of differentiating between endemic typhus and Rocky Mountain spotted fever. The Weil-Felix reaction does not help in this respect and the immunity test in guinea-pigs sometimes fails owing to secondary infections or the existence of non-specific immunity. The results of the experiments showed that the test is of considerable value in differentiating between the two diseases. In endemic typhus the test was positive in all cases (and may remain so up to 5 years or more after the illness), whereas in spotted fever it was almost invariably negative.—I. A. Bengtson and N. H. Topping, *Amer. J. publ. Hlth*, 1942, 32, 48.

Treatment. No specific curative treatment is known and symptomatic treatment is similar to that for epidemic typhus. Destruction of rats is important for prophylaxis. Vaccines of formalised suspensions of the virus from injected guinea-pigs have been used with promising results for immunisation.

Tabardillo. A typhus-like disease prevalent in Mexico presents many features common both to murine and to epidemic typhus. Its exact status is problematical since it has been held that it can be transmitted either by lice or fleas. It is more severe than murine typhus and may be as fatal as epidemic typhus.

Tsutsugamushi Disease. *Syn.* JAPANESE RIVER FEVER, RURAL OR SCRUB TYPHUS, QUEENSLAND COASTAL FEVER, SUMATRA MITE FEVER. An acute infection caused by the *Rickettsia orientalis* (*R. nipponica*, *R. tsutsugamushi*). It is conveyed by the bite of larval mites (*Trombicula deliensis* or *Trombicula akamushi*), mice and other rodents serving as reservoirs of infection. The disease has long been endemic in Japan; it is also common in Malaya, Formosa, Indo-China and the Philippine Islands; the coastal fever of Queensland appears to be identical with tsutsugamushi. In Malaya the disease is not seasonal; it is in a sense occupational, for the victims have all been over country covered with lalang (a tall coarse grass, *Imperata cylindrica*) or other undergrowth. After an incubation period of 11 to 21 days the onset of symptoms is usually abrupt. Following an initial malaise for 24 hours, there is mild headache lasting 2, 3 or, rarely, 5 days, with fever and shivering; occasionally vomiting, and pain in the chest or all over the body. An initial dermal lesion can seldom be found; it may be no more than a papule and the view that the presence of an ulcer is a *sine qua non* of tsutsugamushi can no longer be upheld. The ulcer is merely a stage in the development of a lesion that is first macular, then papular, and only in a few cases progresses to a necrotic ulcer. The papule usually develops and disappears during the incubation period. When present, the ulcer is typical at the

end of the first week of fever; it consists of a black crust surrounded by a contiguous hyperæmic areola. The crust is depressed, round or oval, about 4-6 mm. in diameter; the areola is 4-5 mm. broad with sharply defined outer margin. (An apparently similar dermal lesion is found in *fièvre boutonneuse*, *q.v.*) By the end of the third week the black crust drops off, leaving a well-defined pit. There is associated enlargement and tenderness of lymphatic glands, in axilla, groin and sometimes also epitrochlear and cervical glands. The temperature which is 102°F. for the first few days, rises to a maximum of 104°-105°F. by the 7th to 12th day, falls by lysis to normal or 99°F. by the 13th or 14th day, and is followed by a brief rise to 100°-101°F. on the following day. A rash appears about the 4th to 6th day, but cannot be distinguished in dark-skinned Tamils; in Europeans both macules and papules are present on the chest, abdomen, flanks or limbs and sometimes on the face; the lesions are discrete, dusky-red in colour, fade on pressure, never becoming confluent, and are about 1 to 1.5 cm. in diameter. Fading commences on the third day of eruption and is complete in 6-7 days. Enlargement of the spleen may be due to previous malaria, but may also be due to tsutsugamushi. Deafness, photophobia, mental disturbances, sore throat, hoarseness and furred tongue may be present in many cases.—R. Lewthwaite and S. R. Savoor, *Lancet*, i/1940, 255, 305.

Diagnosis. The presence of a necrotic ulcer, with adenitis, followed by fever, should enable a clinical diagnosis to be made in patients whose occupation is liable to bring them into contact with larval mites. Where the local lesions and adenitis are absent or inconspicuous a Weil-Felix reaction, positive to OXK and negative to OX2 and OX19 strains, would clinch the diagnosis. In Malaya, urban typhus has to be differentiated; here the Weil-Felix reaction is positive with OX19 while male guinea-pigs inoculated with the blood taken early in the disease produce scrotal swelling which does not occur in tsutsugamushi.—Price's *Textbook of Medicine*, 6th Edn., 1941.

Treatment. No specific curative treatment is known and symptomatic treatment is similar to that for epidemic typhus. Prophylaxis consists in avoidance of contact with larval mites.

Trench Fever. This disease was first reported amongst the troops in France in the War of 1914-1918. According to Roche-Lima it is due to *Rickettsia quintana*. The louse has been definitely proved to be the vector, the infection being conveyed by scratching or rubbing the excreta, or crushed bodies of lice, into the abraded skin. It has been shown that the virus in louse faeces can resist drying at room temperature, exposure to sunlight and hot water and soap, and can withstand keeping for at least four months. It is not affected by 20 minutes exposure to dry heat at 80° but is killed in the same period by moist heat at 50°. Thus, clothing, etc., though effectively deloused, is not necessarily disinfected. A man may be entirely free from lice at the time he develops trench fever which he may have contracted from blankets or clothes contaminated with infected louse faeces.

The incubation period varies from 14 to 30 days though it may be shorter if a large dose of the virus has been absorbed. The onset is usually sudden, with headache, dizziness, pains in the legs

and back, and pain behind the eyes. The pain in the limbs is always worse at night, especially in the shins. The temperature usually rises rapidly and may reach 103°–104°F within a few hours of onset of symptoms; during the course of the fever the temperature curve may be extremely erratic (*vide infra*). There is enlargement of the spleen and in bad cases the patient becomes anæmic. In the majority of cases, rose-coloured spots appear on the chest and abdomen during the first few days of fever; they are not raised and disappear on pressure. A peculiar and very constant feature is the rapid alternation of shivering and sweating. Usually within about a fortnight from the onset of the disease the acuteness of the pains subsides though there may be recurrence of pyrexia and of the leg pains for the next week or two. Although trench fever is not a fatal disease it may leave behind it a train of unpleasant sequelæ of which the most important is disordered action of the heart.

The pyrexia calls for special mention. There are two forms, occurring at different stages of the disease: (1) An irregular, remittent and intermittent fever lasting for a period rarely exceeding four weeks. It may be so slight as to escape notice. (2) A definitely intermittent fever, often showing a regular periodicity, and sometimes extending over a period of many weeks. These together constitute the complete pyrexial wave of the disease, but it is important to note that the first form when well marked, may present three types of temperature curve: (a) A short, influenza-like fever wave, lasting about 3 days (b) A similar wave followed on the 6th, 7th and 8th days by a febrile relapse, the interval being afebrile, and which may be followed by an irregular fever; (c) The initial wave may run more or less into the relapse and produce a so-called saddle-back or pseudo-typhoid fever, followed, possibly, by irregular fever. The above are the types recognised by the War Office Investigation Committee.—*W.O. Memo. Med. Dis.*, 1941.

Diagnosis. Diagnosis is difficult though the "spiky" character of the secondary fever, and the shin pains, are highly suggestive. Care must be taken to differentiate it from influenza, malaria, enteric fever, dengue, relapsing fever, undulant fever, rheumatic fever and typhus.

Treatment. This is mainly symptomatic. Aspirin, 20 gr. at night, is the best analgesic, and mustard leaves ease the pain in the shins; vinum colchici has also been found helpful.

Rocky Mountain Spotted Fever. *Syn.* ROCKY MOUNTAIN TICK TYPHUS. A non-epidemic severe typhus-like fever caused by *D. rickettsi* and transmitted by ticks (*Dermacentor andersoni* and *D. variabilis*), the virus being transmitted hereditarily to the eggs. The disease was first recognised in the Rocky Mountain States but is now known to be endemic throughout the U.S.A., a total of 2190 cases having occurred in 37 out of the 48 States during the period 1933–1937. The occurrence of the infection is seasonal, human infections occurring during the tick season, i.e., during the spring and summer months. Those most affected are workers in rural areas. The mortality is from 5 to 20%, though in cases of the severe fulminating type it may be as high as 90%; it is lowest in children and highest in the aged.

The period of incubation is from 3 to 12 days, most commonly 4 to 8 days. The onset is generally sudden and the symptoms similar to those of epidemic typhus, except that there is a greater tendency to hæmorrhage and gangrene. The rash is first seen on

the wrists and ankles, later spreading all over the body and finally to the abdomen where it is always least marked. The fever lasts for two or three weeks and falls by rapid lysis; fulminating cases usually die within 7 to 10 days. One attack confers permanent immunity.

Diagnosis. A positive Weil-Felix reaction may be obtained with *Proteus* OXK, OX2, or OX19, though a significant rise in the titre is often not evident until the fever is subsiding.

Treatment. There is no specific curative treatment and the symptomatic treatment is the same as for epidemic typhus. Prophylaxis consists in the wearing of tick-proof clothing in endemic areas and a careful searching of the body for ticks, iodine being applied locally after their removal. A vaccine prepared from the phenolised emulsion of infected ticks has protective but no curative value and vaccination must be repeated each year.

The vaccine made from the tissues of infected ticks gives a high degree of protection against death, but little protection against attack in areas of highly virulent infection. In areas of infection of low virulence vaccination gives almost complete protection against attack. The only condition in which vaccination is not recommended is when a bite by a suspected tick has already occurred in areas of highly virulent infection.—R. R. Parker, *Amer. J. trop. Med.*, 1941, 21, 369.

The U.S. Public Health Service vaccine, given in two doses of 2 ml. each at an interval of five days, either subcutaneously or intramuscularly, fully protects the majority of persons against the less virulent strains, but the average person is only partially protected against the highly fatal type and the maximum degree of protection conferred is retained for less than one year.—J. G. Hutton, *J. Amer. med. Ass.*, ii/1941, 413.

Sao Paulo Rural Typhus. It is now definitely established that this apparently new disease, which first occurred at Sao Paulo, Brazil, in 1929, is identical with Rocky Mountain spotted fever. It is conveyed from rats to man by the tick, *Amblyomma cajennense*, and has a mortality of 70%.

Fièvre Boutonneuse. *Syn.* MEDITERRANEAN FEVER. This mild, eruptive, typhus-like fever occurs along the Mediterranean coast of France and in Italy, Spain, Portugal, Greece, Rumania, Morocco, Algeria, Tripoli, Egypt and Syria. It is transmitted to man by the bite of a tick, *Rhipicephalus sanguineus*, found on dogs during hot weather. The tick bite usually occurs on parts of the body covered by clothing, and causes a dark brown puncture, described as the "black spot," the discovery and recognition of which is the most important point in diagnosis. The fever may begin sharply, with rise of temperature to 101° or 102°, or slowly, reaching 101° on the third day, the patient complaining of headache, and joint, bone and muscle pains. Pulse regular at about 100, tongue slightly coated, vomiting may occur, some difficulty in swallowing, and injection of conjunctivæ. Rash appears on the second to fourth day, comes out first on the trunk and limbs and spreads in 48 hours to the whole of the skin surface. It forms slightly raised, round or oval maculæ separated by areas of normal skin. These are pink at first, becoming red and then purple. The pharynx is red and slightly swollen, and the soft palate often shows maculæ. Temperature begins to fall when eruption is complete. Spleen and occasionally the liver are increased in size. By the fifth or eighth day fever falls to 99° or 100°, where it remains for 3 or 4 days and then falls by lysis to normal on the twelfth or fifteenth day, when the rash fades and disappears. The disease

has a favourable prognosis with mortality rate of less than 2%, without relapses, and with subsequent immunity.

Diagnosis. This can be confirmed by the Weil-Felix reaction, i.e., by demonstrating the presence of agglutinins against *Proteus* X19 in the patient's blood. The reaction is negative during the febrile period but positive following fall of temperature.

Mild cases of spotted fever cannot be differentiated from *boutonneuse* fever, but in the latter disease a primary sore and adenitis are nearly always present.—G. M. Hass and H. Pinkerton, *J. exp. Med.*, 1936, 64, 601.

Endemic or murine typhus occurs among members of ships' companies on board French men-of-war in Toulon harbour, but these men do not contract *boutonneuse* fever, whereas other people who live on shore contract *boutonneuse* fever and do not get endemic typhus.—Le Chuiton, per *Trop. Dis. Bull.*, 1937, 470.

Tick-Bite Fever. *Syn.* SOUTH AFRICAN TICK TYPHUS. A mild disease occurring in southern Africa, caused by rickettsias transmitted by ixodid ticks, *Amblyomma hebraeum*, *Rhipicephalus appendiculatus*, and *Boophilus decoloratus*. The common dog tick, *Hæmaphysalis leachi* has also been proved to be a vector in all stages—larva, nymph and adult—and there is hereditary transmission of the infection through the egg to the succeeding generation. Hereditary infection probably occurs also in the other species of ixodid ticks. The disease is characterised by a primary sore often having a blackish necrotic centre, swelling of the regional lymph glands and in most cases by intermittent or remittent fever lasting 10–14 days; a maculo-papular rash appears on the third day of illness and when profuse, typically involves the palms of the hands and soles of the feet. All ages, from young infants to old age, are susceptible, though most cases occur in adolescents and young adults and are probably associated with camping and shooting in rural areas. The incubation period is usually one week. In many cases general symptoms are absent, only a primary sore and regional lymphadenitis being found. In moderately severe cases there are lassitude, most prominent in the evening after undue fatigue, feelings of chilliness and slight rigors, headache which may become so severe as to be almost unbearable, feverishness, delirium and visual or auditory disturbances. The mortality rate is about 1%.

Diagnosis. The characteristic features of the primary sore, swelling of lymph glands, and rash in a patient complaining of headache are usually sufficient to establish a diagnosis on clinical grounds. The diagnosis can be confirmed by finding that serum taken from the patient after the tenth day of illness agglutinates one of the strains of *Proteus* used in the Weil-Felix test. Sera from tick-bite fever patients agglutinate *Proteus* OX19 and OX2 and, irregularly, OXK. The serological findings in tick-bite fever resemble in many respects those of the other diseases of the tick-fever group.

Prophylaxis. Avoid contact with ticks by wearing protective clothing when in tick-infested areas and make a careful search for larval ticks, especially on the lower extremities and in the pubic region. Tick-infested dogs should not be allowed in houses. Specific prophylaxis by means of vaccine prepared from tick-bite fever rickettsias grown on chorio-allantoic membrane or yolk-sac of chicken-embryos has not been adequately tested.

Treatment. Treatment is mainly symptomatic, as for other typhus-like fevers. Neosarsphenamine (0.3 to 0.45 g. for an adult) if given early is said to abort an attack after one injection.

"Q" Fever. This is a mild typhus-like disease with a febrile period of 7 to 24 days, occurring among dairy farmers and workers in abattoirs in Australia, first described by E. H. Derrick (*Med. J. Aust.*, 1937, 2, 281). The casual agent is a rickettsia now known as *Rickettsia burneti* which differs from other rickettsias in its failure to produce agglutinins for *Proteus X* strains in the serum. The natural reservoir of this disease is the Queensland bandicoot, and infection is transmitted from one animal to another by the tick *Hæmaphysalis humerosa*.—F. M. Burnet and M. Freeman, *Med. J. Aust.*, 1939, 887.

A rickettsia isolated from ticks (*Dermacentor andersoni*) in Montana, U.S.A., passes Berkefeld filters N and W (which are impermeable to the rickettsias of typhus and Rocky Mountain spotted fever) and is closely related to *R. burneti*. There is complete cross-immunity between the two types of rickettsia in guinea-pigs.—R. E. Dyer, *Publ. Hlth Rep., Wash.*, 1939, 1229.

Both rickettsias are pathogenic for two species of monkeys (*Macacus rhesus* and *Macacus cynomolgus*) and for guinea-pigs and mice. Although the American strain is of greater virulence than the Australian it is concluded that the types from Queensland and Montana should be included in the same species.—F. M. Burnet and M. Freeman, *Med. J. Aust.*, 1939, 887.

Ringworm. The organism of *favus* is *Achorion Schonleinii*; those of *Tinea tonsurans* (RINGWORM OF THE SCALP) and *T. circinata* (RINGWORM OF THE BODY), i.e., non-hairy skin, are *Microsporon Audouini*, *Trichophyton ectothrix*, and *endothrix* (according as the fungus lies outside or inside the hair); that of *Tinea versicolor* (PITYRIASIS) is *Microsporon Furfur*.

Tinea barbae or *Hyphogenic sycosis* (RINGWORM OF THE BEARD) is a common affection of the beard. The common grey coccus inhabiting the upper layers of the epidermis may cause infection and pustulation, but the fungus can be distinguished from this coccigenic variety. Syphilis may also sometimes simulate ringworm of the beard. *Eczema marginatum* is a name for ringworm attacking the groins and axillæ. *Onychomycosis* or ringworm attacking the nails only—not common, but very troublesome.

Staining. For permanent stained sections:—

(1) Soak the hairs in potash solution for 10 minutes. (2) Stain with aniline gentian violet (q.v.) for 1 hour. (3) Absorb excess of stain. (4) Treat with Gram's iodine solution 2 minutes, wash in water. Decolorise with acidified aniline oil (aniline oil 10, nitric acid 1) for 15 to 20 minutes. Treat with aniline oil 1 minute, clarify in xylol, and mount in balsam.

Rapid clinical method of search:—

(1) Soak the hairs in potash solution for 10 minutes. (2) Wash in water to free from alkali. (3) Mount in glycerin or glycerin jelly.

Cultivation of ringworm fungi is possible on all ordinary media, but the addition of glucose or maltose is most helpful.

Diagnosis. The trichophytin test for ringworm. 0.1 ml. of trichophytin (a protein extract of *Trichophyton*) is injected intradermally into the volar surface of the forearm. The reaction may be read at 24 and 48 hours. A positive reaction is generally accepted as indicating that the body is producing antibodies to destroy the fungus.

Scarlet Fever. In 1923, G. F. and G. H. Dick were able to produce scarlet fever in the human subject by infecting the throat with a culture of a hæmolytic streptococcus isolated from a known case of the disease, and in 1924 they demonstrated that the scarlet

fever streptococcus produces a diffusible toxin present in filtrates from cultures which, injected intracutaneously in persons susceptible to the disease, elicits a reaction of cutaneous erythema and inflammation now known as the Dick reaction. It has also been shown by the Schultz-Charlton reaction that the serum of convalescents contains a neutralising antitoxin and further that the serum of convalescents neutralises the Dick toxin when mixed with it and injected into the skin of a known susceptible person. These facts, together with the constant occurrence of hæmolytic streptococci in the throat, strongly support the view that scarlet fever is due to infection of the throat with a hæmolytic streptococcus capable of producing a specific diffusible toxin which is responsible for the general manifestations of the disease, and that the infection is in the first instance local, the general condition being essentially a toxæmia.

The modern conception of scarlet fever is not that of a disease *per se*, but rather of a particular syndrome which may or may not occur in the course of hæmolytic streptococcal infection. The scarlet fever syndrome, i.e., the punctate erythematous rash, the faucial congestion, and the red papillated tongue, is evidence of absorption of a particular exotoxin, the so-called Dick toxin, which is derived in varying quantity from any of the 28 Griffith types of hæmolytic streptococcus pathogenic to man (*Streptococcus pyogenes*).—H. S. Banks, *Practitioner*, i/1939, 693.

Bacteriology. The streptococcus of scarlet fever belongs to Lancefield's Group A; the most frequent types are Types I, II, III and IV, which together are responsible for between 60 and 70% of cases of scarlet fever occurring in Great Britain. There is no serological distinction between scarlet fever strains and hæmolytic streptococci from other lesions in the human subject.

In scarlatinal cases investigated in Edinburgh in 1937, Type I was the most prevalent, but a year later Type IV was most common and produced a more severe infection than Types I and II and was responsible for many of the secondary complications.—H. L. de Waal, *J. Hyg., Camb.*, 1941, 41, 65.

In Roumania, Type X was the epidemic strain; in Sydney, Australia, Types I, IV, XI and XVII accounted for 75% of all scarlatinal cases, whereas Types II and III were never isolated.—*Lancet*, i/1941, 385.

For information concerning the Dick test, Schultz-Charlton test, and methods of prophylaxis and treatment, see Vol. I, pp. 1064 et seq.

Schistosomiasis. *Syn.* BILHARZIASIS. Schistosomiasis is due to infestation with blood flukes of the *Schistosoma* family. Three species are well known. *S. hæmatobium*, which occurs in Africa, Western Asia, etc., *S. mansoni*, which is found in Africa, South America, etc., and *S. japonicum*, which occurs in the Far East—Japan, China, Formosa, and the Philippines.

S. hæmatobium. The adult worms infect mainly the pelvic plexuses and the classical urinary form of bilharzia is the result. The symptomatology varies in proportion to the degree of parasitisation, which in the majority of cases is comparatively light. Irritation and a burning sensation towards the end of micturition are probably the first symptoms observed, followed by urgency, dysuria and hæmaturia. With the increase in pathological changes in the bladder and adjacent tissues further symptoms ensue, cystitis and an ascending pyelitis are commonly evident, and extensive sepsis and disorganisation of the urinary tract and neighbouring tissues bring in their train a variety of sequelæ.

Microscopic examination of the terminal drops of urine will usually reveal the presence of the characteristic terminal spined ova.

S. mansoni. The worms in this infection inhabit the mesenteric veins and the branches of the portal system in the liver. Lesions are found chiefly in the lower portion of the large bowel, more particularly in the sigmoid flexure and rectum. The liver becomes greatly cirrhotic and the spleen is usually considerably hypertrophied, giving rise to the condition known as Egyptian splenomegaly. The symptomatology is exceedingly variable and there is nothing essentially characteristic of the disease. In more severe cases the symptoms range from those of a mild enterocolitis to those of a frank dysentery with irregular fever and severe anæmia. Sigmoidoscopic examination and recovery of the lateral spined ova in the fæces establish diagnosis.

S. japonicum. This helminth parasitises the veins and venules of the mesenteric radicles of the portal system. Its presence in the abdominal organs is usual, and it gives rise to a most grave form of bilharzia. If untreated the disease may be regarded as ultimately a fatal one in every case. Invasion of the mucosa of the small intestine leads to dysentery, slight daily fever, epigastric pain and tenderness, anorexia and loss of weight. The liver and spleen become enlarged and there is enlargement of the mesenteric glands. The fæces become dysenteric in character. Marked interstitial hepatitis and hepatic cirrhosis are later evident, and ascites develops accompanied by diminished urinary secretion. Recognition of the characteristic spineless eggs in the stools leaves no doubt as to the nature of the condition.—A. R. D. Adams, *Med. Pr.*, 1937, 554.

Treatment. Specific treatment is given as early as possible with the object of killing the worms before permanent damage is done to the tissues. The drugs most commonly used, as having a specific lethal action on each of the three parasites, are tartar emetic, emetine, and stibophen. For further details of treatment with these drugs see Vol. I.

Sprue. *Syn.* TROPICAL SPRUE, PSILOSIS, CEYLON SORE MOUTH. Sprue is a disease of unknown ætiology which is endemic in Ceylon, India, Java, South China, the Federated Malay States, the West Indies and the Southern United States; it is rare in tropical Africa.

Adult Europeans and people of mixed European blood living in endemic areas are prone to the disease; both sexes are susceptible, but it rarely affects people under 20 years of age.

In the established disease there is indigestion, flatulence, abdominal distension and febrile diarrhœa, the stools being loose, bulky, of fatty consistency, light-coloured or white, foul-smelling and gaseous. The skin becomes harsh, dry and pigmented, especially on the face and forehead. The tongue is clean but looks red, raw and shiny from atrophy of the filiform and fungiform papillæ. There is thinning of the mucous membrane of the mouth, with inflammation and small ulcers, which makes eating very

painful. There is usually some degree of anæmia, and in severe cases the blood picture may approach that of pernicious anæmia. There is increasing loss of weight, the temperature becomes subnormal and œdema of the feet, cramps, and even tetany may be observed. Paræsthesia is often complained of and tendon reflexes may be absent. Toxic peripheral neuritis is a not uncommon complication.

If left untreated, wasting and debility increase and the patient eventually dies of malnutrition, anæmia or intercurrent disease.

Ætiology. The specific cause is not yet discovered. Biochemical studies point to a general breakdown of digestion and assimilation; there is failure to absorb fats, sugar and calcium, while the hydrochloric acid and pepsin of the gastric juice are diminished and often, as in pernicious anæmia, absent altogether, and the intrinsic factor is missing. Two facts which especially confuse the issue about its ætiology are its remarkable latency (it may declare itself 25 years or longer after return from an endemic area to a temperate zone) and its liability to relapse after many years of freedom from symptoms. Theories of its origin have implicated diet, worms (*Strongyloides stercoralis*), yeast infection (*Momilia psilosis*), dry rot in houses and the fæces of termite ants. Many of the changes in the epithelium of the small intestine, formerly considered specific, are ascribed to agonal and post-mortem changes. No readily demonstrable changes can be seen in the intestinal tract, but on microscopical examination there is usually evidence of chronic inflammation. The essential lesion appears to reside in the ileum which is generally much distended with gas, its walls being atrophic and diaphanous.

The absence of a distinctive pathological picture brings sprue into line with idiopathic steatorrhœa, pernicious anæmia and pellagra. The sprue syndrome can be mimicked by other dissimilar processes involving the ileum and interfering with its normal functions.

A review of all the known facts would seem to indicate that in the disease-group typified by the pernicious anæmia-pellagra-sprue syndrome the essential causative lesion is localised somewhere in the gastro-intestinal tract, and is of such nature and extent as to interfere more especially with absorption of one or several of the vitamins essential to life. The inability of the absorptive mucosal surface to perform its proper functions is best expressed by the term "inefficiency." We can therefore apply this idea to parts of the intestinal tract and speak about "gastro-duodenal inefficiency," "jejuno-ileal inefficiency," "ileo-cæcal inefficiency," and so forth. The hypothesis is therefore set forth that in order to produce the syndrome characteristic of each disease the essential lesion should involve some particular and limited tract, thus:

A. Pernicious anæmia syndrome: "gastro-duodenal inefficiency."
1. Primary pernicious anæmia. Naturally occurring disease (absence of intrinsic factor). 2. Secondary pernicious anæmia. Artificially produced disease subsequent to partial gastrectomy.

B. Sprue syndrome: "jejuno-ileal inefficiency." 1. Primary sprue. Naturally occurring disease. *Typical:* tropical sprue. *Atypical:* non-tropical sprue. 2. Secondary sprue. Artificially produced disease subsequent to gastro-jejuno-colic fistula, gastro-jejunostomy and short circuit, neoplasm of small intestine, mesenteric tuberculosis, lymphadenoma, etc. 3. Celiac disease.

Congenital absorption defect in small intestine proceeding to idiopathic steatorrhœa in adults.

C. Pellagra syndrome: "ileocæcal inefficiency." 1. Primary pellagra. Naturally occurring disease (absence of vitamin B₃ complex). 2. Secondary pellagra. Artificially produced disease—damage to ileum and mainly to large intestine by chronic bacillary or amœbic dysentery, alcohol and surgical interference.—P. Manson-Bahr, *Trop. Dis. Bull.*, 1941, 38, 128; see also *Lancet*, i/1940, 317 and 356, and *Med. Annu.*, 1942, 35.

Differential Diagnosis. This includes cœliac disease, pellagra, pernicious anæmia, tropical macrocytic anæmia, gastric carcinoma, mesenteric tuberculosis and chronic pancreatitis.

Cœliac disease (idiopathic steatorrhœa) and tropical sprue are two relatively common diseases distinct from one another. Cœliac disease is a disease of children, tropical sprue of adults. Megacolon is common in older patients in the former and is rare or hardly known in the latter. Rickets and deformed bones are found in cœliac disease alone. Established cœliac disease is fatal in 40% of cases, while tropical sprue has an extremely good prognosis under modern conditions.—T. I. Bennett and C. Hardwick, *Lancet*, ii/1940, 381.

According to T. E. Hess Thaysen, cœliac disease of children, idiopathic steatorrhœa of adults in temperate climates (non-tropical sprue) and sprue of the tropics are fundamentally identical. Infantile sprue and adult sprue bear the same relation to each other as do cretinism and myxœdema. The osteoporosis of the child has its analogue in osteomalacia in the adult, and the low blood calcium and phosphorus, the type of anæmia, the aphthous stomatitis, xerophthalmia and nyctalopia are changes recognisably due to avitaminosis.—F. M. Hanes and A. McBryde, *Arch. intern. Med.*, i/1936, 1.

Treatment. The essentials of treatment are: (1) The institution of alimentary rest by appropriate dietary; (2) the treatment of megalocytic anæmia if present; (3) the reinforcement of demonstrable deficiencies by such means as HCl, calcium salts, etc. A milk diet is recommended, the feeds being given in small quantities 2-hourly, commencing with 3½ and increasing gradually up to 7 pints daily. Fairley has introduced graded high protein, low fat, low carbohydrate diets, the ratios of the three fundamental foodstuffs being as 1·0 : 0·3 : 1·3 and the energy values varying from 600 to 3000 calories. Red meat is the main source of protein advocated. The anæmia responds rapidly to the administration of commercial liver extract in adequate quantity.—G. C. Low and N. H. Fairley, *Price's Practice of Medicine*, 5th Edn., 1941.

The administration of adequate doses of liver extracts effective in pernicious anæmia, especially by parenteral injection, is fundamental in the treatment of sprue and its macrocytic anæmia. The accessory use of iron is indicated for certain patients. It is emphasized that adequate doses of liver extract are as important in controlling the manifestations of the alimentary tract as in promoting the formation of blood in cases of sprue.—W. B. Castle *et al.*, *Arch. intern. Med.*, 1935, 56, 627.

Twenty-four well-marked cases of tropical sprue treated by nicotinic acid in doses of 150–300 mg. daily and 7 with the addition of riboflavin. The most striking effect was upon the glossitis and rapid return of taste-sense. The tongue returns to normal within 3 or 4 days, but in glossitis and angular stomatitis of advanced sprue riboflavin 3 mg. daily appears necessary. The effect upon the intestinal symptoms is slower but impressive. Diarrhœa ceases within 4 days, and stools are normal in size and colour in 2 or 3 weeks. The mentality and outlook of the patient and texture and appearance of the skin are favourably influenced, but most satisfactory of all is the absence of meteorism and flatulency, usually an intractable feature. Vitamin B₃ does not play any active part in hæmatopoiesis and it is necessary to continue parenteral liver therapy. It is probable that nicotinic acid in a "reserve" dose of 50 mg. daily has to be continued for 3 months after disappearance of all active symptoms.—P. Manson-Bahr, *Trans. R. Soc. trop. Med. Hyg.*, 1941, 34, 347.

The treatment which has been employed at Millbank for many years and has proved more satisfactory than any other is based on the administration of milk, vitamins of fresh fruit and yeast, liver products and later liver. The diet progresses by four-day gradations and all milk must be taken with a teaspoon. The dietary treatment is prefaced by a dose of castor oil sufficient to clear out the fermenting material, but avoiding a severe purge. (A complete series of diets for nineteen four-day periods is given.)—W.O. *Memo. Med. Dis.*, 1941.

Staphylococcal Infections. The staphylococcus is a round, Gram-positive coccus which divides irregularly into masses. The staphylococci are grouped roughly into three types according to their pigment production; *Staph. pyogenes aureus*, which produces a golden-yellow growth, is the common cause of various suppurating conditions such as boils, abscesses, carbuncles and other skin conditions, and is found in osteomyelitis and septic endocarditis; *Staph. pyogenes albus*, which produces a white growth, is commonly found on the skin and in the nose and throat; *Staph. pyogenes citreus* produces a lemon-yellow growth and is only feebly pathogenic.

Staph. pyogenes aureus. A spherical coccus about 0.9μ in diameter, growing irregularly in clusters or masses. It is Gram-positive and grows rapidly in all ordinary media at room temperature, though much more rapidly at body temperature. On agar a stroke culture is at first yellow, and then bright orange. It liquefies gelatin. Hæmolysin is produced in culture and many strains produce an enzyme, coagulase, which coagulates blood plasma.

Staph. pyogenes albus. Similar to the above, but cultures are white. May or may not show hæmolysis. Colonies of the *albus* type may occur as a variant in cultures of *Staph. aureus*.

Staph. pyogenes citreus. Less frequently met with and differs in colour of cultures, these being lemon-yellow. It is usually far less virulent than the two above. Does not usually liquefy gelatin.

Staph. cereus albus and *Staph. cereus flavus* are wax-like on gelatin. Growth does not liquefy gelatin.

Staphylococci are very sensitive to crystal violet, acriflavine and brilliant green, and growth is inhibited by these dyes in high dilutions.

Recent work shows that staphylococci can be differentiated into two groups using coagulase production as the criterion. The coagulase-producers are pathogenic (*Staph. pyogenes*); the non-coagulase producers are non-pathogenic and should be called *Staph. saprophyticus*. Further sub-division, if required, could be based on pigment, or for *Staph. pyogenes*, on slide agglutination.—R. W. Fairbrother, *Brit. med. J.*, i/1940, 543.

To demonstrate coagulase one drop of a broth culture of *Staph. aureus* is added to citrated rabbit plasma and incubated at 37° for 3 hours.

Fisk's Method is as follows: Citrated human plasma is diluted 1 : 10 with normal saline and 0.5 ml. of the dilution is placed in each of two test-tubes. To one tube 0.125 ml. of a 12-hour broth culture is added; the second tube is kept as a control. Both tubes are incubated at 37° and examined after 30 minutes and at intervals for 6 hours. Clotting usually occurs in less than 1 hour.

Toxins. Some strains of *Staph. aureus* and *Staph. albus* produce a toxin when grown on Walbum's medium. This toxin is hæmolytic, leucocidic (destroys white blood cells), dermonecrotic (causes necrosis of tissues), and is rapidly fatal to experimental animals in intravenous injection. Two types of staphylococcal hæmolysin have been described: α -hæmolysin—rapidly lyses rabbits and sheep red blood cells at 37° , and β -hæmolysin slowly lyses sheep red blood cells at room temperature. Human red blood cells are acted on by β -hæmolysin, but are only slightly affected by α -hæmolysin. (For staphylococcus enterotoxin, see *Staphylococcal Food Poisoning*, p. 876.)

Walburn's Medium. Meat extract from ox-heart with 0.5% peptone (Witte), 0.2% potassium dihydrogen phosphate and 0.03% magnesium sulphate. pH 6.8. Grow culture in atmosphere containing 20-25% CO₂.

Staphylococcus Antitoxin. Standard, B.P. The standard preparation is a quantity of dried staphylococcus antitoxin kept at the National Institute for Medical Research, Hampstead.

Unit. The unit is the specific neutralising activity for staphylococcus toxin contained in such an amount of the standard preparation as the Medical Research Council may from time to time indicate as the quantity exactly equivalent to the unit accepted for international use.

Methods of Comparison. Various methods of comparison are described in the *B.P. Add. I*, in all of which a test toxin is first prepared and the test dose determined by finding the amount of the toxin just sufficient to produce a given effect when mixed with 1 unit, or with a simple fraction of 1 unit, of standard antitoxin. Unknown samples of antitoxin are assayed by making mixtures with the test dose of the test toxin and testing these as before. The tests applied are: (1) the power of the toxin to hæmolyse the corpuscles of the rabbit; well-washed corpuscles are added to the toxin-antitoxin mixtures; according as the mixture contains excess or insufficient antitoxin, there is no hæmolysis or complete hæmolysis; (2) the power of the toxin to cause skin reactions in guinea-pigs or rabbits; (3) the power of the toxin to kill mice after (a) intravenous, or (b) intraperitoneal injection.

Streptococcal Infections. *Streptococcus pyogenes* occurs in chains of varying length, the cocci being slightly larger than staphylococci (1 μ in diameter). Virulent strains have a capsule. Involution forms are seen in cultures, some of the cocci being as much as double size. The organism is Gram-positive. It grows on ordinary media, but best on media containing blood or serum. On solid media recently isolated pathological strains produce "matt" colonies; avirulent strains produce "glossy" colonies. It ferments lactose, saccharose, glucose and salicin, and usually has a strong hæmolytic action (on blood agar). The organism can survive for a time in air and dust.

Varieties. Besides *pyogenes* the group of streptococci includes types which are normal inhabitants of the mouth, throat and intestine. The streptococci are classified according to the appearance of colonies on blood-agar plates. A wide zone of hæmolysis round the colonies is characteristic of β or "hæmolytic" streptococci; *S. pyogenes* belongs to this class. Colonies with a greenish coloration—due to methæmoglobin formation—are characteristic of the α or "viridans" streptococci; some of the normal inhabitants of the mouth and throat belong to this class. Colonies in which there is no obvious change in the blood agar are found in the third group γ which includes many of the streptococci from the throat, mouth and intestines. The faecal streptococci, sometimes called "enterococci," are oval or lanceolate and in pairs or short chains; they usually ferment mannitol and æsculin; resist 60° for 30 minutes; growth not inhibited by bile salt; produce H₂S; some liquefy gelatin; virulence low.

Anærobic (micro-ærophilic) streptococci are usually non-hæmolytic; fœtid gas formation in cultures is common. Anærobic streptococci are sometimes present in septic and gangrenous conditions of the skin and they are sometimes present in puerperal sepsis.

Serological Classification. The hæmolytic streptococci are classified into a number of groups based on a precipitation reaction with a carbohydrate substance specific to the members of each group. (For details see R. C. Lancefield, *J. exp. Med.*, 1933, 57, 571; R. Hare and L. Colebrook, *J. Path. Bact.*, 1934, 39, 429 and R. C. Lancefield and R. Hare, *J. exp. Med.*, 1935, 61, 335.)

Group A contains most strains from human infections—sore throat, scarlet fever, cellulitis, erysipelas, puerperal sepsis; they are β -hæmolytic and produce a soluble hæmolysin; ferment trahalose but not sorbitol. The members of this group represent the typical *S. pyogenes*; all the Griffiths types (*vide infra*), except 7, 16, 20 and 21 are included in this group.

Group B strains are primarily associated with bovine mastitis (*S. agalactiæ*). Some strains from the human throat and vagina and some from puerperal infection belong to this group.

Group C are associated with infections in horses (*S. equi*) and guinea-pigs. β -Hæmolytic—occasionally in human infections.

Group D occur in dairy produce and in the human intestine; a hæmolytic variant of the enterococcus.

Groups F and G are found in the human throat, but Group G is also responsible for infections in the dog.

Other groups so far identified are E, H, K, L and M, but there is no evidence that they are associated with human infections.

Griffith's Types. Thirty serological types of hæmolytic streptococci of epidemiological significance have been separated by agglutination and agglutinin-absorption tests. The commonest types are 1, 2, 3, 4, 6, 8, 9 and 10. The anti-sera for agglutination are prepared by injecting killed cultures of known-type strains into rabbits and purifying the sera by absorption tests with other types. Cultures of the organisms are made in serum-broth, the supernatant fluid is decanted and the deposit of streptococci used for slide-agglutination tests.—F. Griffith, *J. Hyg., Camb.*, 1935, 34, 542.

Studies on puerperal infection have shown that Group A streptococcus is seldom present in the genital tract ante-partum, and that its presence there in the puerperium does not necessarily mean a severe infection. Streptococci belonging to Groups B, C, D and G may be present in the vagina, both ante-partum and in the puerperium.—R. P. Lancefield and R. Hare, *J. exp. Med.*, 1935, 61, 335.

Group B streptococci were found in three fatal cases of post-partum infection.—R. M. Fry, *Lancet*, i/1938, 199.

Among 1163 cases of puerperal sepsis investigated, 48 cases yielded streptococci belonging to groups other than Group A. 16 were Group B, 12 Group C, 12 Group G, 6 Group E, and 2 were unclassified.

Streptococci other than Group A were recovered from 43% of post-abortion streptococcal infection, and from 9% of post-partum streptococcal infection.—A. M. Ramsay and M. Gillespie, *J. Obstet. Gynec.*, 1941, 48, 569.

Group A hæmolytic streptococci produce two serologically distinct varieties of hæmolysin, one designated streptolysin O on account of oxygen sensitivity, the other streptolysin S on account of extractability in serum. The response of animals to experimental infection with whole cultures of A hæmolytic streptococci was a rise in titre of the neutralising antibodies, antistreptolysin O (A.S.O.) and antistreptolysin S (A.S.S.).—E. W. Todd, *Brit. J. exp. Path.*, 1938, 19, 367, and *J. Hyg., Camb.*, 1939, 39, 1.

Examination of the antistreptococcal titres of 1346 sera from adolescents including healthy subjects as well as various clinical groups showed the mean titre for normal controls was 79; in 82.3% the titre was less than 125 units. Increased titres were noted in scarlatina (mean 300 with maximum titres in 3rd or 4th week), in pharyngitis due to *S. hæmolyticus* (mean 263), the active

phase of acute rheumatism (mean 444) and the inactive phase of acute rheumatism (mean 210).—C. A. Green, *J. Path. Bact.*, 1941, 53, 223.

Hæmolysis. The medium used by Schotmueller is two parts human blood (or rabbit) and five parts melted agar, but 5 to 10% of blood is better.

The technique employed by Andrewes and Christie is as follows: Ordinary peptone broth containing 10% horse serum of pH 7.13, incubated at 37° for 24 hours (or 48 hours for weakly growing strain) is centrifuged till apparently clear. One drop of dense suspension of horse erythrocytes (well washed in normal saline) is added to each 10 ml. tube of broth—the fluid should be opaque and bright red on gently shaking. Incubate at 37° and examine after 20, 30 and 60 minutes and then every hour.

Cultivation. Hæmolytic streptococci may be recovered, often in profuse culture, from blood agar plates containing 1 in 500,000 gentian violet when cultures on plain blood agar are negative. Of 300 swabs, 68 were positive on blood agar, while 98 were positive on gentian violet. If proteus or many *Ps. pyocyanea* are present in material for culture hæmolytic streptococci may be recovered by covering the inoculated plate with melted agar and sterilising the surface of this with absolute alcohol or by inoculating a blood agar plate containing phenol 1 in 1000.—A. E. Francis, *Lancet*, ii/1941, 159.

By the simple method of spreading a small quantity of gentian violet (2 drops of a 1 in 3000 solution) over half of the culture plate of blood agar after it has been planted it is possible to isolate a few streptococci from the midst of a multitude of staphylococci, just as it has been shown to be if the gentian violet is incorporated in the medium. The spreading should start where the inoculum is lightest and proceed towards the heavier inoculum. Penicillin (6 drops of crude culture filtrate) and potassium tellurite (2 drops of 1 in 1000 solution) can be used similarly.—A. Fleming, *Brit. med. J.*, i/1942, 547.

A 1 in 500,000 gentian violet blood agar has a selective bacteriostatic action separating all streptococci from all staphylococci, other indifferent gram-positive cocci, diphtheroids and the aerobic spore-bearing bacilli. Most of the non-pathogenic flora of air and dust can be eliminated by the employment of a gentian violet medium.—L. P. Garrod, *Brit. med. J.*, i/1942, 290.

Syphilis. *Treponema pallidum* (*Spirochaeta pallida*), the causative organism, varies from 6 to 14 μ in length, averaging 8 to 10 μ . It is much more attenuated than the majority of spirochaetes, having a maximum thickness of 0.3 μ , has from 6 to 12 twists, forming a close, regular and narrow spiral; it is actively motile. Its movements are characteristic and best seen under the dark ground; they consist of a rapid rotation round the longitudinal axis, bending or flexions of the spiral, and a slow forward or backward movement. It is readily destroyed by heat at 52°, by drying, and by antiseptics; it dies rapidly in water.

Cultivation. Serum water, to which a piece of sterile rabbit tissue (preferably kidney or testicle) has been added, is inoculated from the artificially infected testicular tissue of the rabbit (not from human lesions). The serum (in test tubes) is rendered suitable for anaerobic cultivation by a layer of paraffin oil poured upon its surface. After the first cultivation strict anaerobiosis is not essential—the organism can be subcultured on to solid media such as gelatin or agar. The first growths are usually contaminated by other bacteria. Two methods are suggested for separating these from the spirochaetes: (1) To grow the spirochaetes through filters which retard the passage of other organisms, or (2) a method depending on the fact that in stab cultures the spirochaetes grow away from the line of puncture into the surrounding medium, while other bacteria fail to do so. Noguchi states that spirochaetes cultivated by these methods are pathogenic in so far that after inoculation into the rabbit's testicle they produce characteristic histological changes and are found growing freely in the infected tissue.

STAINING METHODS

Giemsa's Stain. Dissolve 0.3 g. of Azur II eosin and 0.08 g. of Azur II in 25 ml. of anhydrous glycerin at 60°. Add 25 ml. of absolute methyl alcohol at the same temperature. Allow to stand overnight, and filter. Immediately

before use dilute one part of stain with 10 to 15 parts of distilled water or buffered solution. If intense staining is desired, dilute with 1 in 1000 solution of potassium carbonate.

Fix the film in absolute methyl alcohol for 1 to 5 minutes. Dry. Cover the cover-slip with diluted stain and leave for 15 to 30 minutes. Wash in distilled water until the film has a pinkish tinge; dry and mount. To demonstrate *Treponema pallidum* stain for 2 to 12 hours.—Stitt.

Long and diligent search is necessary in looking for spirochaetes stained by this method. It imparts to the spirochaete a distinctly reddish-violet tinge, similar to that of the neighbouring leucocyte nuclei (the Romanowsky chromatin stain), whilst the bacteria come out blue.

Indian Ink Method (Burri). The method requires no special apparatus. A platinum loopful of secretion from a sore is placed on a slide and mixed with an equal quantity of distilled water and an emulsion of Indian ink. The whole is mixed and spread on the slide like a blood film, allowed to dry and examined with oil-immersion lens. The ink produces a dark background and the objects stand out white.

Fontana's Silver Impregnation Method. (a) *Fixing fluid:* Acetic acid 1 ml., solution of formaldehyde 20 ml., water 100 ml. (b) *Mordant:* 5% tannin in a 1% aqueous phenol solution. (c) *Silver Solution:* Silver nitrate 0.25% in distilled water. In use, a minute quantity of ammonia is added until there is a distinct turbidity (avoid excess).

Dried films (not fixed by heat) are fixed in (a) 1 minute, the fluid being dropped on and renewed once or twice. The preparation is then washed thoroughly, solution (b) is dropped on the film, heated until steam rises and allowed to remain about $\frac{1}{2}$ minute. Again wash in water, drop on solution (c), heat as before and allow to remain about $\frac{1}{2}$ minute. Finally wash and dry. *Treponemata* are dark brown or black, and are easily found.

Congo Red. Stained films acidified with dilute hydrochloric acid as relief staining for bacteria and spirochaetes.

A small drop of 2% aqueous congo red solution is placed on the slide and a very small quantity of the bacterial culture or of the exudate to be examined is mixed with it. The drop is then spread out into a tolerably thick film. Allow to dry and wash the slide with 1% hydrochloric acid in absolute alcohol and dry in the air, or films may be spread and stained afterwards and treated with acid. Examine with oil-immersion lens. The background will appear as a rule uniform. Bacteria vary somewhat in relation to the dye. Mostly they are clear, sharp and quite transparent, but some will take up the dye and appear as ill-defined bluish-black bodies—this is seen chiefly in old cultures of gram-negative organisms.

Nigrosine stains the background leaving the spirochaetes colourless. The method is simple and apparently as accurate as the dark-field technique and can be used on dry preparations.—Dienst and Sanderson, *Amer. J. publ. Hlth.*, 1936, 26, 910.

Levaditi's Method for staining spirochaetes in tissues. Thin, small pieces of tissue are fixed in formaldehyde solution for 24 hours, washed in water for 1 hour and immersed in alcohol (96–98%) for 24 hours. They are then placed in 1.5% AgNO_3 solution in a dark bottle and kept in the incubator for 3 days. After washing in water for half an hour, the pieces of tissue are placed in a reducing mixture composed of pyrogallol 4 g., solution of formaldehyde 5 ml., water to 100 ml., and kept in a dark bottle for 48 hours at room temperature. After washing well in water, the tissue is dehydrated in increasing strengths of alcohol and embedded in paraffin from which sections are cut in the usual way.

Examination of Unstained Specimens by Dark-Ground Illumination. *Tr. pallidum* is found below the surface in the lymph only and should be sought at the margin of the lesion. It cannot be detected in the centre of an ulcerated or necrosed area where the saprophytic spirochaetes may be seen in large numbers. The organism is found in the largest numbers in mucous plaques, is constantly present in varying numbers in primary untreated chancres, and is usually detected in the papular syphilide and in scrapings from a recently removed enlarged syphilitic lymphatic gland.

General or local treatment has a marked effect on the number of *treponemata* found, and the organisms tend to disappear after a few weeks from the site of the primary inoculation, even without treatment. Antiseptics must not have been previously applied to the sore.

The margin of the chancre, papules, or mucous plaque should be gently scraped till blood just begins to exude. The surface is now dried with a swab of plain sterile gauze, and then a little blood or serum expressed by decomposition or by bandage. A small drop of this is removed with a platinum needle and mixed with a drop of distilled water on a thin glass slide. A thin cover-glass is now pressed down firmly, so that only a thin layer of fluid remains between the slide and cover-glass. A drop of immersion oil is placed below the slide and on the cover-glass. The condenser must first be accurately centred. This can easily be done with a low objective (1 in. or $\frac{1}{2}$ in.) by means of concentric rings scratched on the surface of the condenser.

Any artificial light can be used. Concentrate the light on the centre of the microscope mirror. After the slide has been placed in position so that there is a layer of oil between the ultramicroscope and the under surface of slide, and after the object is focussed, the ultramicroscope must be racked up or down and the mirror adjusted till bright illumination with dark background is obtained.

Tr. pallidum seen thus is an extremely fine silvery spiral. If so focused that only the summits of the spirals are illuminated the organism looks like a series of bright dots, not unlike a chain of streptococci. It preserves its spiral form during rest.

Comparison with other Treponemata. *Tr. refringens* under dark-ground illumination is seen to shoot rapidly backwards and forwards in a straight line and when not rotating so actively is often seen to squirm its way by corkscrew movements, pushing blood corpuscles, bacilli, etc., aside. Serum obtained by swabbing is preferable for examination to scrapings. Stitt says: "While the rotary movement of *Tr. pallidum* is rapid, it does not move across the field with the speed of other spirochaetes. Thus *Tr. refringens*, commonly present in genital ulcerations, quickly traverses the field and shows more widely separated spirals. The *pallidum* shows a continuity of its spirals while in motion, but when at rest often shows the appearance of a series of silvery dots or dashes. Many individuals show a bend in the long axis."

Tr. buccalis, *Tr. refringens*, *Tr. balanitidis* are much larger with wider and more open spirals. *Tr. refringens* has only 3 to 5 turns and is usually blunt at either or both ends. The only spirochaetes very like the specific organism are: (1) *Tr. dentium*, found in carious teeth, which is shorter (5 to 10 μ) and coarser, 5 to 15 spirals, the wave length the same as *Tr. pallidum*, but depth of wave is considerably less; (2) *Tr. pertenuis* Castellani (yaws); (3) *Tr. pseudopallida* Loewenthal (ulcerated cancers). In the last two the spirals are not quite so deep or regular, and in the case of *Tr. pertenuis* the ends are often twisted into rings or loops.

Noguchi's Method of Diagnosis of Syphilis. (*Distinguish from the Noguchi modified Wassermann.*) Boil two parts of the cerebrospinal fluid with 5 parts of a 10% solution of butyric acid in normal saline for a few seconds, then add one part of normal sodium hydroxide and again boil briefly. A flocculent or granular precipitate is obtained on standing (in parasyphilitic affections) due to presence of a globulin. The test distinguishes general paralysis from other forms of insanity not associated with meningo-encephalitis.

Examination of Dried Serum. The blood is collected in the usual way in a bent Wright's Tube, and allowed to coagulate. A definite quantity of the serum is pipetted off and allowed to dry on blotting paper. This can then be treated with normal saline and made up to its original volume for conducting the test.

WASSERMANN REACTION

The reaction was elaborated by Wassermann on the principle of the Bordet-Gengou reaction (1901). The Bordet-Gengou reaction is that which occurs when antigen, specific antibody and complement are exhibited together. As Wassermann was not able to use a culture of the *Tr. pallidum* he made use of an extract of syphilitic *fœtus tissue* rich in spirochaemata. Subsequent work showed, however, that the reaction was not a true Bordet-Gengou reaction, was not truly specific, and did not depend on an interaction between the spirochaemata and a specific anti-spirochaemal antibody, but was due to an altered condition in the serum of a patient during the active stage of infection, whereby, on the addition of various lipoidal substances an alteration occurred which caused complement to be interfered with in its activity. Modifications using

"antigen" extracted from normal organs are now employed to a large extent. For the reaction are required:

(1) *Antigen*, which is usually an alcoholic extract of normal heart to which some cholesterin has been added.

(2) *A hæmolytic system*, usually anti-sheep, requiring sheep's red cells and rabbit serum from a rabbit immunised against sheep's red cells.

(3) *Complement* obtained in guinea-pig serum.

(4) *Serum of patient and controls* of normal and syphilitic sera. These sera should be inactivated by heating for 30 minutes at 55° to 56° before use.

The only reliable test is when the constituents for the reaction have all been derived from separate sources and can be accurately standardised. The so-called rapid clinical methods are valueless.

Washed Sheep Corpuscles are prepared from the fresh blood by removing fibrin by clotting—rapidly stirring at the time of drawing from the animal. Centrifuge with a powerful centrifuge and pipette off the serum. Add normal saline solution and again centrifuge several times, and finally dilute with normal saline solution making approximately a 10% suspension of the corpuscles.

Methods of carrying out the Wassermann Reaction. The methods of performing the test are legion, but the principles involved are but slightly different from those in connection with the complement fixation test in general. Three agents are involved in the actual reaction, antigen, patient's serum and complement. When these have been allowed to react for a suitable period (1 hour at 37°) or overnight in the icebox, sensitised red cells are added and the mixture incubated for 1 hour at 37°. This determines the presence or absence of hæmolysis, indicating the presence or absence of unabsorbed complement. To establish a positive reaction it must be shown: (a) that the patient's serum alone absorbs no complement, or at the most but a small fraction of it; (b) that the same is true of antigen; (c) that the mixture of serum and antigen absorbs an amount of the complement greatly in excess of the sum of the small amounts absorbed by the individual reagents separately.

There are three variables involved in the reaction—antigen, serum and complement. Quantitative results may be obtained by keeping two of these constant and varying the third.

Topley and Wilson prefer the method whereby the antigen and complement remain constant and the variable third is the patient's serum. The serum is tested with dilutions of 1:2.5, 1:5, 1:10, 1:20 and 1:40. This gives a direct measure of the concentration of the Wassermann body in the serum under test. The serum control is put up with the largest amount of serum employed in the actual test, and it is necessary to standardise the complement.

NOTATION. With the large number of methods employed there are, of necessity, many notations. Some use + + + +, + + +, + +, +, + -, and - to indicate strongly positive, positive, moderately positive, weakly positive, doubtful, and negative results respectively. Others employ numerals from 0 to 4 to indicate the amount of hæmolysis in the various tubes of the reaction; thus Kolmer uses 4 4 4 4 4 to indicate a strong positive, 4 4 0 0 0 to indicate a moderate positive, and 0 0 0 0 0 to indicate a negative. Nevertheless it is desirable that a brief written note be added such as—"definitely positive," "doubtful" or "definitely negative."

Varying amounts of blood serum are required for the carrying out of the W.R. according to the method employed. 1 ml. of blood or 0.25 ml. of serum is the minimum quantity but 5 to 20 ml. of blood and an ample supply of serum enables the test to be repeated if necessary and confirmed. Generally speaking the blood may easily be obtained by venepuncture or simply by "milking" the finger or lobe of the ear.

For further details of reliable methods of carrying out the Wassermann Reaction see *Spec. Rep. Ser. med. Res. Comm.*, 1929, No. 129; *Rep. Minist. Hlth.*, 1932, No. 67; *J. Path. Bact.*, 1934, 39, 521.

Parasyphilitic Conditions in Relation to the Reaction. The W.R. in blood and spinal fluid is often valuable in confirming a diagnosis of early tabes, but as a guide to progress and further treatment it is unreliable, as one frequently finds actively progressing tabes with a history of syphilitic infection despite a negative reaction in both blood and fluid.—C. P. Symonds, *Modern Technique in Treatment*, Vol. II, p. 40.

CEREBRAL SYPHILIS. A definitely positive W.R. indicates infection—a negative result is not always of value. In tabes, a negative reaction in the blood

is by no means uncommon during a quiescent phase; even in the spinal fluid, the reaction is sometimes negative. In cerebral syphilis, when the disease is progressive, a +W.R. in the blood can almost always be obtained. In the cerebrospinal fluid the reaction may be + or —.—G. Riddoch, *Lancet*, ii/1923, 3.

Noguchi gave the following figures as indicating the percentage of positive W.R. as determined by various serologists: Primary syphilis, 69·8; secondary syphilis, 89·4; tertiary, 78·1; hereditary, 94·5; cerebrospinal, 47·6; general paresis, 88·1; tabes, 62·66. With cerebrospinal fluid general paresis gave 90, tabes 56·2 and cerebrospinal syphilis 19.

Lange's Colloidal Gold Test, see *Cerebrospinal Fluid Examination*, p. 704.

OTHER SEROLOGICAL TESTS

The phenomenon of precipitation with syphilitic sera was observed soon after that of complement fixation and many attempts have been made to apply this phenomenon in the development of a precipitation test for syphilis. Of these those of Meinicke, Sachs and Georgi, Vernes, and Dreyer and Ward gained some recognition, though none received wide acceptance. Kahn in 1921, by the application of certain governing principles and by paying particular attention to the standardisation of the antigen and technique, developed a test which has attained wide use throughout the world. Apart from its apparent specificity and high sensitivity the test possesses the practical advantage of being completed within a few minutes after mixing the ingredients.

Since the development of the Kahn test a number of other precipitation methods have been proposed by different authors. Among the best-known of these are the Kline and Hinton tests. The problem of determining which is the best method for general use is a difficult one, but investigations conducted under the auspices of the Health Organisation of the League of Nations (1928 and 1930) and of the U.S. Public Health Service (1935) proved the Kahn test to be the most dependable.—Stitt.

Kahn Test. To a 1 in 5 alcoholic beef-heart extract add 4 mg. cholesterol to each ml., a similar amount of extract being retained as a non-cholesterolised antigen control. Dilute the alcoholic antigen with normal saline solution (0·85%) in proportion of 1 to 2, and the cholesterolised antigen in the proportion of 1 to 3. Pipette 1 ml. of alcoholic antigen into a small test-tube, add 2 ml. of salt solution, and mix rapidly. The resultant opalescent mixture is ready for use. Dilute the cholesterolised antigen similarly, using 3 ml. of salt solution; this has a tendency to precipitate, and is best kept in the incubator when not in use. Dilutions should be freshly made and not used until $\frac{1}{2}$ hour after dilution.

Place in each of 2 agglutination tubes 0·3 ml. of undiluted inactivated patient's serum. To the first tube add 0·05 ml. of diluted non-cholesterolised antigen, and to the second the same quantity of diluted cholesterolised antigen; agitate vigorously. Strongly positive sera may show definite precipitation, particularly with the cholesterolised antigen. To bring out precipitation in weaker sera, place test-tubes overnight in incubator at 37°. Read results next morning as follows:—(1) Precipitation consisting of one or more large clumps is denoted by + + + +; (2) a large flocculent precipitation by + + +; (3) moderate-sized flocculi or granules by + +; (4) small-sized flocculi or granules by +; (5) fine flocculi or granules by \pm ; (6) negative precipitation by —.—Stitt.

Comparison of Kahn and Wassermann Reactions. Of 9 tests applied simultaneously to 800 sera the Wassermann was the most deficient in sensitivity (66%), with the Sachs-Georgi next, the best being the Meinicke reactions with a sensitivity of 81·7 to 90·3%. The only completely specific test was the routine Kahn. Of the 6 tests applied simultaneously to 380 fluids the most sensitive was the Kahn, whilst the Wassermann was much less so. In view of the fact that the Wassermann test on blood is the most difficult, the least sensitive, and not the most specific, one can but deplore that there should be such a desperate clinging to the Wassermann even to-day, especially in public medicine. Even for spinal fluid examinations the Kahn is vastly superior.—J. E. Nicole and E. J. Fitzgerald, *Lancet*, i/1934, 652.

Kline Test. Ordinary microscopic slides are washed in soap and water and rinsed, allowed to remain in 95% alcohol for a short time, dried and flamed. Four paraffin rings (with an inside diameter of 11 mm. to 12 mm.) are made on one surface by transferring a small amount of hot paraffin on a stiff wire (gauge 19) wound with thread (or hat wire) bent to the form of a circle.

The antigen and antigen dilution are prepared as for the Kahn test. The antigen titration likewise is done as for the Kahn test. The antigen dilution should be made up just before pipetting the sera. Some antigen dilutions have been found to work only within 15 minutes of their preparation. The serum is obtained as for the Wassermann test, and heated to 56° for 30 minutes.

Into each ring 0.06 ml. of the undiluted serum to be tested is delivered from a pipette. It is advisable to work with not more than twelve sera (three slides) at a time. After all the sera are pipetted, 1 drop of Kahn's antigen dilution (0.015 ml.) is allowed to fall into the serum in each ring. After all the antigen is pipetted, the small amount in each ring is evenly distributed by rapidly stirring the mixture with a tooth pick. The slides are then placed below a humidor cover consisting of a wooden lid, 16½ in. by 4 in. by 1½ in. inside diameter, with a moistened blotter fastened in place with thumb tacks, and allowed to remain at room temperature for 10 minutes. The first slide is then removed, rocked for about 60 seconds and read immediately. The readings are made through the microscope (16 mm. objective, 10 or 12.5 eyepiece) with the light cut down as in studying urinary sediments, and recorded in terms of pluses according to the size of the clumps. The test should be done in duplicate, different antigens being used.—B. S. Kline and A. M. Young, *J. Amer. med. Ass.*, i/1926, 929.

Chief value of Kline test is in later stages of syphilis under treatment; in cases, e.g., interstitial keratitis, where it is especially important that syphilis should be excluded; and as a general supplement to Wassermann reaction. It is relatively simple, rapid and inexpensive and only a very small quantity of serum is required. The antigen-emulsion retains its efficiency for a relatively short time only—hence the test is suitable only for use in laboratories where large numbers of sera are examined at one time.—W. V. MacFarlane and J. Gorman, *Brit. med. J.*, i/1935, 469.

Laughlen Test. Use antigen as for Kahn test; saturate with a fat stain, e.g., Scharlach R or Sudan III; 0.5 ml. antigen and 1 to 2 mg. of stain in test tube plugged with cork covered with tinfoil to reduce evaporation; warm to 50° in water-bath, three to four minutes, shake; add 2 drops of compound tincture of benzoin, shake; dilute at once with 5 ml. 1-5% saline which must have been previously warmed to 50°. Add saline to antigen and mix by pouring back and forth four or five times. Replace in water-bath for 2 minutes, cool. The colour of the agglutination reagent should be reddish white, rather than deep red. (If any particles other than a few heavy portions of undissolved stain are observed throughout the solution it should be discarded.) Set aside for 20 minutes, decant from undissolved stain. Reagent must be sensitized to react in one minute with strongly positive sera and must be free from agglutination for at least 10 minutes with negative sera. The sensitivity of reagent is adjusted by adding (if necessary) about 1 drop 9% sodium chloride solution per ml. (or 1 or 2 drops saturated ammonium sulphate solution to each 10 ml.) of reagent.

Two drops of reagent are placed on a glass slide, and a large loopful of the patient's serum or citrated serum (free from red blood cells) or two loopfuls of spinal fluid are added. Mix by jarring or tilting repeatedly and observe at short intervals by holding slide near a strong light and looking through it toward a dark object. At first the mixture appears as a white turbid fluid, in which small reflecting granules are seen on tilting the slide. In non-syphilitic specimens the appearance does not alter appreciably until drying is about complete. Slightly coarser granules may then be observed, but there is no true clumping or agglutination. Excess drying should be avoided. With positive sera coarse particles soon develop if the slide is sufficiently jarred or tilted backwards and forwards. These particles increase in size, and become distinctly red in colour, and tend to accumulate at the margins of the drops. Positive readings can often be made within one minute, but negative readings should not be recorded until at least ten minutes have elapsed. The time required for the reaction to become visible is an index of the degree of positivity of the specimen.—G. F. Laughlen, *Canad. med. Ass. J.*, 1935, 179.

The use of mastic instead of benzoin and adjustment of the pH to about 6.2 to 6.5 by a phosphate buffer solution, is recommended by F. Rappaport and F. Eichhorn, *Lancet*, i/1943, 426.

Hinton Test. Consists of an "agglutination" by syphilitic sera of a suspension of cholesterol in glycerinated hypertonic saline containing a trace of the alcohol-soluble, ether-insoluble extractives of beef muscle. An economical, simple, and

highly sensitive test, but should be reserved as a supplementary test to the Wassermann.

It has been shown that in a group of 143 patients with treated syphilis, the Hinton has proved to be twice as efficacious as the Kahn and three and one-half times the Wassermann. In detecting unsuspected syphilis in a group of approximately 5000 cases of cancer, tuberculosis, and pregnancy, the Hinton was found to be nearly twice as efficacious as the Wassermann. False positive Hinton's are shown to be extremely few in number.—A. W. Cheever, *New Engl. J. Med.*, i/1936, 113.

Modifications of the Hinton test which give accurate readings in one hour.—J. A. V. Davies, *J. Lab. clin. Med.*, 1937, 22, 959.

Sachs-Georgi Reaction. Mix a 1 in 5 alcoholic extract of heart muscle (beef, human, or guinea-pig), with 2 parts of alcohol, and to 10 ml. of this add 0.45 ml. of 1% cholesterol solution. (The proportions may have to be varied.) Dilute with 5 parts of normal saline solution, taking care that no precipitation occurs and that the dilution does not become cloudy. Inactivate patient's serum by heating for $\frac{1}{2}$ hour in a water-bath at 55° and dilute with 9 parts of normal saline solution. Place 1 ml. of the diluted serum in a test-tube and add 0.5 ml. of the diluted antigen. As antigen control, mix 0.5 ml. of diluted antigen with 1 ml. of normal saline solution (0.85%) and as serum control mix 1 ml. of diluted serum with 0.5 ml. of normal saline solution and alcohol mixed in the proportion of 5 to 1. A known positive and a known negative serum are used for control. Incubate test-tubes for 18 to 20 hours.

Interpret with an agglutinoscope after 18 to 24 hours. The antigen control should be absolutely clear, and if the serum control shows any precipitation repeat the test. Distinct light particles against the dark background indicate positive reaction—negative sera are entirely clear or slightly opalescent. Strongly positive reactions show up within 2 or 3 hours in the incubator and can be read microscopically.

Cerebrospinal fluid should be used undiluted in 1 to 1.5 ml. amounts.

Meinicke's Modification of this test uses an alcoholic, non-cholesterolised horse-heart extract for antigen.

Meinicke later used a larger amount of balsam of tolu, and ox-heart instead of horse-heart muscle, dilutions being made with 3.5% salt solution, containing sodium carbonate, and the reaction is carried out at a temperature of 20°. When positive, the mixture becomes clear—slightly positive, opalescent—negative, turbid.

Dreyer and Ward's Reaction (Sigma Reaction). Instead of five variables there are only two. An antigen which is a mixture of an acetone-free alcohol-soluble heart extract and cholesterol is used. Flocculation after 9 hours at 37° is a + reaction.

Vernes Flocculation Test. Depends on a flocculation reaction between the patient's serum and a special reagent, "Perethynol," prepared from horse-heart muscle by means of ethylene chloride and alcohol. A suspension of the reagent in saline is flocculated in the presence of syphilitic antibody and the degree of haemolysis ascertained by a special photometer, the results being given quantitatively in definite figures.

Ide Test. This is a colour test. One drop of blood, serum, or cerebrospinal fluid is mixed with saline on a hollow glass slide, the antigen is added and the result read with a lens or low-power microscope. The antigen is an alcoholic extract of ox-heart containing cholesterol, benzoin and certain dyes. The results obtained are stated to be in close agreement with the W.R.—S. Ide and T. Ide, *J. Lab. clin. Med.*, 1936, 21, 1190.

Tetanus. Tetanus is an infective disease which is characterised by tonic spasm of the masseter and other muscles, caused by the action on the central nervous system of a toxin produced by the growth of tetanus bacilli in a deep, penetrating wound. The incubation period is usually from 5 to 14 days.

Bacteriology. *Clostridium tetani* is a long slender bacillus 4 to 5 μ long and 0.3 to 0.8 μ broad, weakly motile, and forming drumstick spores which are resistant to heat and antiseptics, though they are killed by heating for 5 minutes in the autoclave. The bacillus grows anaerobically on ordinary

media; liquefies gelatin slowly; does not coagulate milk and does not ferment carbohydrates. The optimum temperature for growth is 37°. Fildes' medium containing a peptic digest of blood (see p. 913) is suitable for the anaerobic culture of *Cl. tetani*.

The tetanus bacillus produces a soluble toxin which is one of the most powerful poisons known and which can be prepared by growing the bacilli anaerobically in broth and filtering after 14 days.

It is often impossible to detect the *Cl. tetani* in stained films made from the exudate of wounds; the diagnosis may be confirmed by producing tetanus in mice by the subcutaneous injection of the filtrate from an anaerobic culture.

Tetanus Antitoxin. Standard, B.P. The standard is a sample of dried antitoxin kept in the National Institute for Medical Research, Hampstead.

Unit. The unit is the specific neutralising activity for tetanus toxin contained in such an amount of the standard preparation as the Medical Research Council may from time to time indicate as the quantity exactly equivalent to the unit accepted for international use. The British unit is the same as the international unit, but the American unit has twice the amount of activity represented by the international unit.

Method of Standardisation. To estimate unknown samples of antitoxin, a test toxin is first prepared. Tetanus toxin, unlike diphtheria toxin, can be prepared as a stable dry powder by growing *Bacillus tetani* in broth in the absence of air; the organisms in the broth are killed and the broth is filtered. When this sterile filtrate is saturated with ammonium sulphate, a precipitate is formed which can be dried and powdered. This can be preserved in sealed ampoules. Mixtures of toxin and the standard antitoxin are first made in order to find the smallest quantity of toxin which when mixed with 0.2 unit of antitoxin and injected under the skin of guinea-pigs or mice, causes death in four days. This dose is called the L₁ dose. The injected volume is 4 ml. for a guinea-pig or 0.5 ml. for a mouse. The L₁ dose of the test toxin having been determined, the sample of unknown antitoxin is then mixed in varying amounts with the L₁ dose of the test toxin and these mixtures are injected into guinea-pigs or mice, in order to find the mixture which kills the injected animals in 4 days. The amount of unknown antitoxin in this mixture contains 0.2 unit.

Tick Fever. The different varieties of tick-borne relapsing fevers include: (1) Central African tick fever due to *Borrelia (spirochaeta) duttoni* transmitted by *Ornithodoros moubata*; (2) Somaliland tick fever transmitted by *O. savignyi*; (3) Persian and North-West Indian relapsing fever caused by *Sp. persicum* and transmitted by *O. papillipes*; (4) Spanish relapsing fever attributed to *Sp. hispanicum* and transmitted by *O. erraticus*; (5) Central American relapsing fever due to *Sp. venezuelense* transmitted by *O. venezuelensis*; (6) Panama relapsing fever due to *Sp. neotropica* transmitted by *O. talaje*.

Ross and Milne (1904) first showed the so-called African tick fever to be caused by a spirochaete of closely similar character to that of relapsing fever, but bacteriologically it is more convenient to keep the two diseases separate—associating tick fever with *Sp. duttoni*. Clinically, the fever closely resembles relapsing fever, but the periods of fever are somewhat shorter, rarely lasting more than two or three days, and the relapses are more numerous. The organisms are much fewer in the blood than in the European relapsing fever. Morphologically they are almost the same. The tick-borne infection is not transmissible by lice.

The transmitting agent, *Ornithodoros moubata*, infests rest-houses on the route of travel, hiding in the crevices of floors and walls and feeding at night. The female transmits the spirochaete to its ova. Natives suffer severely in childhood, but develop immunity later.

Through the bite of ticks from Nyasaland, collected in the hut of a native in whose house cases had occurred, Leishman was able to infect a monkey. The spirochaetes appeared in the blood of the animal on the sixth day and it died on the thirteenth day. From the monkey, transmission was possible to mice.

See also Relapsing Fever and Typhus, pp. 937 et seq.

Trichiniasis. *Syn.* TRICHINOSIS. A disease acquired by the consumption of uncooked or inadequately cooked pig-flesh contaminated with living larvæ of the nematode *Trichinella spiralis*. The larvæ are liberated in the gastro-intestinal tract during digestion of the meat. Within a few days they mature and mate, after which the females discharge new larval forms into the wall of the small intestine. The young larvæ enter the blood stream and are carried to all parts of the body. Some lodge in voluntary muscles where they become encysted and remain alive for many years; others lodge in various organs where they die within a few weeks. Live larvæ present in the muscles later do not produce symptoms or signs of disease.

Symptoms usually appear from seven to twelve days (extremes 3 to 20) after ingestion of infested meat and include slight fever (up to 102°F.), increased pulse rate, oedema of the eyelids, chemosis and suffusion of the conjunctival blood vessels. These signs seldom persist for more than a few days. Subungual hæmorrhages occur in severe cases, and when present are said to be almost pathognomonic. Hypotension is common during the febrile stage. Pulmonary features are common and occasionally hæmoptysis occurs. When migration of larvæ commences myositis of the tongue, laryngeal and intercostal muscles and of the diaphragm occurs, giving rise to difficulty of swallowing, speech and respiration; the muscles of the jaws, arms, legs and abdomen may also be involved, with stiffness and pain; the affected areas are tender and hard to the touch. Abdominal pains, distension, diarrhoea or constipation are less common, and occasionally there are nervous symptoms, such as focal paresis, or signs of general inflammation suggestive of meningitis or encephalitis. From 1 to 5% of cases terminate fatally with evidence of myocarditis, encephalitis, or both.

Diagnosis. Adult trichinellæ are not easy to find in the faeces, but during the first three or four weeks of illness larvæ can sometimes be found in the blood. Five ml. of blood is laked by diluting with 50 ml. of water, centrifuged, and the sediment examined under low power. The larvæ are approximately $100\ \mu \times 6\ \mu$, and have a granular appearance.

Relative and absolute increase in eosinophils is an almost constant accompaniment of the stage of larval dissemination. During the first two or three weeks of illness eosinophils usually comprise 15 to 40% of the total circulating leucocytes. Variation in the percentage is often noted from day to day. In moribund patients, or when there is a pyogenic complication such as pneumonia, there may be a temporary suppression of these cells.

Later, when there is muscle pain, biopsy of a piece of the affected muscle will often reveal precystic or encysted larvæ.

Infestation with *Trichinella spiralis* causes the development of specific antibodies which may be demonstrated by a skin test or by a precipitin test with trichinella antigen (a saline suspension of powdered larvæ of the parasite).

Leucocytosis occurs in a primary wave, reaching its peak at about the third week, and a secondary wave reaching its peak at the eleventh week. The eosinophils diminish or may disappear within the first three days from onset of symptoms. Thereafter they increase in primary and secondary waves, coinciding with those of the general leucocytosis. Neutrophils diminish during the waves of general leucocytosis, and increase as the eosinophilia diminishes. Monocytes increase with the first diminution in the eosinophilia.—B. L. Della Vida and S. C. Dyke, *Lancet*, ii/1941, 69.

Seventy-eight cases were reported in Birmingham in February, March and April, 1941. The main criteria of diagnosis (which were present in all cases, except one) were the oedema of the eyelids and eosinophilia. Frontal or orbital headache was common at the outset, and was occasionally the most distressing symptom. Conjunctival injection, slight or severe, was the rule. No subungual hæmorrhages were seen. A characteristic onset was pain between the shoulder blades spreading up the back of the neck to the occipital region, later affecting the limbs, and in severe cases becoming generalised. The pain was severe, aching or cramping in quality, worse on movement, especially after rest. The results of the intradermal test were inconsistent and indicated that the test is not by itself a reliable criterion of diagnosis.—L. J. Bacon, *Brit. med. J.*, ii/1941, 909. (For reports of other outbreaks in England and Wales see J. H. Sheldon, *Lancet*, i/1941, 203; R. H. H. Jolly, *Proc. R. Soc. Med.*, 1941, 34, 593; P. B. Beeson, *ibid.*, 585; L. P. Garrod and D. Maclean, *Brit. med. J.*, i/1941, 240.)

Intradermal Test. 0.1 ml. of a 1:10,000 dilution of antigen is injected into the skin of the forearm and an equal quantity of normal saline is injected 5 cm. distally as a control. A positive response may be delayed (evident after twelve to twenty-four hours) or immediate. The delayed reaction consists of redness and induration, similar to a positive tuberculin reaction, and may be elicited as early as the end of the second week of illness, but is usually present for a few days only, after which the response is of the immediate type. This develops during the third or fourth week of illness, and can be elicited for many months, sometimes years, subsequently. It consists of a wheal and erythema (sometimes erythema only) and appears within ten minutes after the injection, beginning to fade in fifteen to twenty minutes. The skin reaction is relatively specific. With good preparations of antigen, positive reactions have been obtained in 90% of clinical cases; false positives are rare. A positive response may be exhibited for several years after an infestation. Hence a positive reaction does not necessarily indicate recent infestation. A negative response in a patient who has been ill for four weeks or longer is strong evidence against the diagnosis of trichiniasis.

Precipitin Test. Useful when the response to an intradermal test is equivocal. Undiluted blood serum is added to an equal volume of antigen diluted 1:200 or 1:500, and incubated for an hour at 37°. Precipitins usually develop at about the same stage of the disease as that at which the intradermal reaction becomes positive.—P. B. Beeson, *Lancet*, ii/1941, 67.

Prophylaxis. The public should be warned to cook thoroughly all pig-meat, whether in the form of sausages, pork, ham or bacon (curing by smoking and salting is ineffective), and all made-up cooked foods, prepared commercially and containing pig-flesh, should be submitted to adequate heat treatment before sale. Garbage or offal used for pig-feeding should be thoroughly cooked.

Treatment. No specific treatment is known, but every effort should be made to expel the adult worms. Good results have been claimed from the use of stibophen.

Tropical Ulcer. A gangrenous ulceration of the skin and subcutaneous tissues of unknown ætiology, resulting in the formation of sloughing ulcers of great chronicity. It is met with

in damp steamy jungle in the tropics. The lower limbs are generally involved and a history of preceding trauma is the rule. Fusiform bacilli, and a spirochæte, *Treponema schaudinni*, are commonly present in the ulcer. The condition is directly transmissible by inoculation of ulcer material from man to man. Generally the ulcers persist for months, a factor delaying healing being inadequate epithelial proliferation, even after a healthy granulation tissue base has formed.

The *Treponema schaudinni* is considered to be identical with Vincent's spirochæte, which is found with fusiform bacilli in ulcerative lesions of the mouth.

Ætiology. On one of the islands of the Admiralty Group the distribution of the disease was found to be correlated with native dietary. Two groups of people were distinguished: the one subsisted largely on sago, the other upon taro. The mean percentage of incidence of tropical ulcer among the two groups was 5.3 and 2.5 respectively. The higher incidence among the sago eaters is believed to be due to their diet being rich in carbohydrates, low in fat, poor in protein, and deficient in vitamin B₁, which acts by lowering the reduction potential of the tissues so that it falls below that of the invading organisms. Neither deficiency of vitamin A nor lowered serum calcium was considered a causative factor. The reservoir of the fuso-spirochæta organisms is believed to be the mouth; the mode of transmission, expectoration, with soiling of the clothes and floor and consequent infection of wounds on the lower extremity. Tropical ulcer reproduced in 76% of twenty-one attempts, using as experimental animals rats previously fed for some weeks on a diet low in protein and fat and deficient in vitamin B₁, the inoculum consisting of a saline emulsion of ulcer scrapings, instilled into denuded areas of skin after traumatization by cautery.—F. W. Clements, *Med. J. Aust.*, ii/1936, 615.

The calcium deficiency does not appear to be in the diet, but seems to be due to the diet, faulty absorption probably being the factor. One of the most important considerations is the quality of the protein in the diet. Ulcers are much more common in vegetarians than in those who include animal substance in their diet. The vegetarian diet, as consumed by the African native, is much too bulky. Digestion of protein, fat and calcium may be greatly interfered with. The most important prophylactic measure, which would go far to prevent the appearance of the ulcer syndrome, is to include meat, eggs, fish, or fowl in the diet, and to reduce the amount of vegetable matter.—A. A. Forbes Brown, *J. trop. Med. Hyg.*, 1935, 215.

Treatment. Calcium chloride injections have been given (15 grains in 10 ml. distilled water daily intravenously) in some 500 cases with remarkable results. Without exception every trace of offensive odour disappears in 3 or 4 days and within 10 days the ulcer is clean.—L. J. A. Loewenthal (Uganda), *Lancet*, ii/1932, 889. See also G. A. Stephens, *ibid.*, 1026.

A satisfactory and surprisingly successful method has been found in excision and skin graft, seeds being the surest and easiest of application, combined in suitable cases with Thiersch grafts. Average time for complete epithelialisation of the area 13 days.—C. James, *ibid.*, 1095.

Cleanse the ulcer by removing necrosed tissues, wash regularly with weak tepid solution of potassium permanganate, and, after drying, powder with a mixture of 100 parts each iodoform and boric acid with 20 parts of bismuth subnitrate, protecting by gauze bandages and daily dressings against reinfection.—S. Golovine, *Pr. med.*, 1933, 2102.

Fifty cases of tropical ulcer in being or in formation were selected for estimation of the blood-serum-calcium. The values found showed that the level of the serum-calcium is not a factor in the pathogenesis. Twenty-three out of the fifty cases were observed until they healed. The rates of healing were found to bear no relation to the level of serum-calcium. It is concluded, therefore, that intravenous injection of calcium salts in the treatment of tropical ulcer has no rational basis.—M. Ellis, *Trans. R. Soc. trop. Med. Hyg.*, Nov. 1936, 387.

Swabbing daily with a mixture of copper sulphate and phenol very effective in 2000 cases (in Assam) when other application had proved valueless. Subsequent dusting with iodoform and bismuth subgallate.—C. McGuire, *Brit. med. J.*, ii/1935, 842.

Trypanosomiasis. *Syn.* SLEEPING SICKNESS. Sleeping sickness is the terminal stage of human trypanosomiasis, a disease due to *Trypanosoma gambiense* or *Trypanosoma rhodesiense*, transmitted by the bite of the tsetse fly, *Glossina palpalis*. The disease is endemic in those regions where the tsetse fly abounds and occurs in Central and Western Equatorial Africa, particularly along the Congo river and its tributaries.

The disease begins as an irregular remittent fever with rapid pulse and increased respiration. It may be accompanied by a patchy erythema and localised œdema, particularly about the eyes and joints; hyperæsthesia may be present especially over the tibia. Lymphadenitis develops, most frequently in the posterior cervical glands, and there may be slight splenic enlargement. This is the first phase, known as trypanosome fever, and it may end in recovery; this phase is invariably seen in Europeans but is sometimes absent in natives. A long period may then elapse, lasting for months, or even years, before the central nervous system becomes involved and the characteristic symptoms of the stage of sleeping sickness appear; usually, however, this second phase appears within four to eight months and once it is established the prognosis in the untreated patient is practically hopeless, death occurring within a year. The earliest symptoms of this second phase are dullness, malaise, lethargy and insomnia; the patient complains of headache, walks with a slow, shuffling gait, and exhibits tremors of various kinds. Later he becomes increasingly drowsy and morose and is continually dropping off to sleep. In the final stages all the symptoms become more pronounced and he becomes a helpless invalid, eventually dying of coma or exhaustion.

T. rhodesiense infection is more serious than that caused by *T. gambiense*, running a course of only a few months, most patients succumbing despite treatment. (According to Wenyon, the distribution of *T. rhodesiense* is very limited, namely, to the districts east and west of Lake Nyasa, and in Northern Rhodesia, Nyasaland, the south-east corner of Tanganyika, and the north-east corner of Mozambique.)

Natives of the infected areas appear to have a greater resistance to the disease than Europeans, but the latter seem to recover better.

Three types of sleeping sickness as seen in Nigeria are described. The most common (over 95% of cases) is a mild form in which equilibrium is maintained though the patient is in poor physical condition with resistance lowered by other infections. In the second type toxæmia is the salient feature and œdema common, and patients die without any obvious involvement of the nervous system. In the third, patients die in the characteristic sleeping sickness stage.—H. M. O. Lester, *West African med. J.*, 1938, 10, 2.

Classification of Trypanosomes. It is now generally accepted that *T. rhodesiense* is a species distinct from *T. gambiense*. There has been considerable controversy regarding the relationship of *T. rhodesiense* to *T. brucei* (*vide infra*). The lack of infectivity of *T. brucei* for the human subject has been

repeatedly proved by inoculation of volunteers. Bruce and his co-workers were of the opinion that *T. rhodesiense* is a strain of *T. brucei* which has adapted itself to man and this view is now widely held.

Several different species of trypanosomes are associated with infections in animals. Nagana, or tsetse-fly disease of horses, is due to *T. brucei* transmitted by *Glossina morsitans*; the disease occurs in Africa and also affects other equidæ, dogs and cattle. *T. vivax* and *T. congolense* infect domesticated animals in Africa. Mal de Caderas, a South American disease of horses is due to *T. equinum*. The organism of Surra, a disease of domesticated animals, including camels, in various parts of the world, is *T. evansi* and is transmitted mechanically by *Stomoxys* and *Tabanus* flies. Dourine occurs in horses and is due to *T. equiperdum*, transmitted by coitus.

Morphology of *T. gambiense*. Its length is between 15 and 30 μ . There are various forms—a short and broad which has no flagellum, a long thin form with flagellum, and an intermediate form. The nucleus is central and the kinetoplast at a point a short distance from the posterior end. Undulating membranes are of moderate width and not greatly convoluted. Granules of volutin may or may not be present in the cytoplasm. *T. rhodesiense* is indistinguishable morphologically from *T. gambiense* in human blood.

Transmission. In man it is transmitted from the sick to the healthy by a tsetse fly (usually *Glossina palpalis*). In the stomach of this fly the trypanosome multiplies by fission.

Human trypanosomiasis never spreads in the absence of glossina and this is the only insect in which the trypanosomes are known to develop cyclically. The virulence of the strain appears to be entirely independent of its transmissibility. A consideration of the factors which may influence the transmissibility shows that of four possible explanations of inhibitory effect—the fly, the climate, the host, and the trypanosome itself—the transmissibility of a trypanosome by a glossina is a function inherent in the trypanosome itself. Different strains of *T. gambiense* show great differences in cyclical transmissibility by *G. palpalis*, and it is justifiable to infer that at any stage of infection of man by *T. gambiense* the transmissibility of the trypanosome by *G. palpalis* may be lost. Its transmissibility diminishes when the strain is introduced into a sheep or goat and after some months in these animals it loses its transmissibility altogether and it is improbable, therefore, that these animals play any important part as reservoirs of this trypanosome; calves also are a negligible factor. *T. gambiense* may lose its transmissibility quite suddenly on transfer from one host to another by cyclically infected *G. palpalis*.—H. L. Duke, *Final Rept. League of Nations Int. Com. on Human Trypanosomiasis*, 1928.

Diagnosis. Trypanosomes may be found in thick blood films stained with Leishman or Giemsa stain. They are not, as a rule, numerous and better results may be obtained by examining centrifuged citrated blood. Smears obtained from gland juice may show the trypanosomes when they cannot be found in the blood. In the later stages of the disease the trypanosomes can often be found in the cerebrospinal fluid and recently they have been demonstrated in material obtained by sternal puncture. Inoculation of susceptible animals such as rats or guinea-pigs is often an aid in diagnosis.

Red-cell Adhesion. Trypanosomes, immune serum complement and human red blood cells are incubated together. The red cells become firmly adherent to the trypanosomes if the serum is homologous. Standard concentrations of red cells and trypanosomes must be used for constant results.—H. C. Brown and J. C. Broom, *Trans. R. Soc. trop. Med. Hyg.*, 1938, 32, 209.

Staining. Staining is best conducted with Leishman's Stain. First pour on to the film and allow to stain half a minute, then add twice the volume of distilled water and allow to stain further half an hour. Wash in distilled water and dry in customary manner. Other methods of staining are with thionin blue, methylene blue, Giemsa's stain and Borrel's blue.

Manson recommended the examination of the blood when the temperature is high; it is well to centrifuge as the trypanosomes accumulate in the leucocyte layer above the red corpuscles.

Laveran's Method. Prepare thin blood films, and fix in absolute alcohol 5 to 10 minutes. The following are required:—

- (1) Solution:—Borrel's blue (see p. 925).
- (2) Aqueous solution of eosin, 1 per 1000.
- (3) Solution of tannin 5%, or, better, a solution of "Tannin Orange."

Mix just before use: No. 1 solution 1 ml., No. 2 solution 4 ml., distilled water 6 ml. Stain in a flat dish, film downwards, for 5 to 20 minutes—5 to 10 minutes is enough in most cases. Wash in water and treat with tannin for a few minutes. Wash in water and then in distilled water. If precipitate found on the preparation, wash in clove oil and brush off with xylol.

Cultivation. The N.N.N. medium (see p. 915) is suitable for the growth of trypanosomes. Cultures of *T. gambiense*, *T. rhodesiense* and *T. brucei* may also be obtained with Ponselle's medium (*q.v.*), using the same sodium chloride concentration. Rabbit serum may be replaced by monkey serum for cultivation of pathogenic trypanosomes of African mammals. When inactivating the media for the cultivation of pathogenic trypanosomes in the higher mammals it is well to increase the temperature from 70° to 75°.

Trypanosomes cannot exist in a medium contaminated by bacteria. Efforts to obtain positive cultures from blood and cerebrospinal fluid of little value as a diagnostic method. The complement-fixation test should furnish more fruitful results than the precipitin test in identifying the blood found in the alimentary tract of glossinæ.—M. Maximo Prates, *Final Rept. League of Nations Int. Com. on Human Trypanosomiasis*, 1928.

The best results in the cultivation of the pathogenic trypanosomes were obtained with a medium consisting of equal parts of citrated human blood and Ringer solution containing 0.6% sodium chloride, to which was added 0.5 g. per litre of cholesterol.—P. Brutsaert and C. Heurard, *per Trop. Dis. Bull.*, 1937, 530.

Ponselle's Medium. Sodium chloride 0.8 g., Witte's peptone 2 g., gelatin 2 g., normal sodium carbonate solution 1 ml., distilled water to 100 ml. Heat rapidly on water-bath and sterilise in autoclave at 110° for thirty minutes. Cool and add equal volume of rabbits' serum when medium for primary culture is required and defibrinated rabbits' blood for subcultures. Distribute in quantities of 3 ml. in test-tubes and inactivate by keeping for thirty minutes at 56°.

Prophylaxis. A dose of 2 g. of suramin administered to an adult may be expected to confer protection against *T. gambiense* and *T. rhodesiense* for at least three months; and the protection may last longer.

Treatment. The routine treatment in N. Nigeria now consists of three 1 g. doses of suramin or Antrypol, followed by five 2 g. doses of tryparsamide. Toxic symptoms after tryparsamide occurred only when filtered and boiled water was used in place of distilled water.—H. M. O. Lester, *Trans. R. Soc. trop. Med. Hyg.*, 1939, 32, 615.

Simultaneous injection of different compounds in doses individually insufficient to produce a cure gave excellent results. Best mixture was Antrypol and tryparsamide.—A. Sice and F. Torresi, *Bull. Soc. Pathol. exot.*, 1938, 31, 710.

4:4-diamidino-stilbene produces rapid amelioration of sleeping sickness in half the time required for tryparsamide; it is not suitable in cases in which the protein content of the cerebrospinal fluid is above 0.05%. Intravenous injection may produce a fall in blood pressure with alarming symptoms which soon pass off. The drug may also be given intramuscularly.—C. Bowesman, *Ann. trop. Med. Parasit.*, 1940, 34, 217.

For further references to treatment, see under the individual drugs in Vol. I.

Tuberculosis. *Mycobacterium tuberculosis*, the causative organism of tuberculosis, is a long, slender rod, straight or slightly curved, with rounded ends. It is about 2.5 to 3.5 μ in length and 0.3 μ in breadth, though longer forms, up to 6.5 μ , occur in the tissues and in cultures; involution and branching forms are occasionally met with. It is non-motile and Gram-positive and stains with difficulty, often with a beaded appearance. When stained it is not decolorised by 20–25% sulphuric acid ("acid-fast") nor by alcohol ("alcohol-fast").

The bacilli are present in the sputum in large numbers when the process is acute but are relatively scanty or absent in chronic

forms of tuberculosis. On account of its waxy coating the bacillus is very resistant to drying and is capable of living for several months in dried sputum. It is killed by heating at 60° and is rapidly killed by direct sunlight.

DIAGNOSIS

Direct Methods of Diagnosis. Clinical evidence of the existence of tuberculosis, however complete, must never lack the confirmation of bacteriological proof and in all doubtful cases the isolation and recognition of the bacillus is of vital importance. Since the only radical proof of the existence of the disease is the demonstration of the bacillus in material obtained from the patient, attention must first of all be directed to this investigation. By comparison with this demonstration even the most definite reaction to one or other of the tuberculin tests, or the most strikingly positive result with the complement-fixation method, is untrustworthy. In cases of suspected phthisis every effort should be made to secure sputa, and the single, isolated plug of mucus, which is often expectorated in the early morning, should not escape attention. In little children, sputa, as such, are usually absent, but if vomiting occurs in association with lung disease the vomit should be searched for fragments of sputa. In suspected disease of the kidney or urinary tract the urine must be collected carefully and submitted to examination. The faeces should be scrutinised in doubtful cases of the lung, peritoneum or bowel. If any puncture-fluid is obtainable, as from the pleura, spinal theca, or a joint, this is valuable for investigation in any patient suspected of tuberculosis. The very different significance to be attached to a positive as against a negative result in all these examinations must never be lost sight of; the former affords proof of the existence of a tuberculous lesion, the latter gives at most a presumptive evidence against it.—*Price's Textbook of Medicine*, 6th Edn., 1941.

The presence of tubercle bacilli in the urine does not indicate that progressive renal tuberculosis will inevitably follow. Tubercle bacilli are not commonly found in the urine even in cases of far advanced pulmonary tuberculosis and may be demonstrated repeatedly in some cases in which tuberculous infection has not been suspected. It is a wise procedure to test for the presence of tubercle bacilli in the urine in cases in which leucocytes and erythrocytes are consistently present.—W. H. Ordway and E. M. Medlar, *J. Amer. med. Ass.*, 1942, 119, 937.

Examination of Sputum. Films may be spread from the purulent portion, or the bacilli may be concentrated by the antiformin method.

To stimulate an active cough reflex a swab may be inserted into the throat, or the patient may be made to sniff the vapour of a small quantity of essential oil of mustard. Intralaryngeal injection by syringe of a few drops of a weak solution of sodium bicarbonate to which has been added a little hydrogen peroxide has also been advocated. The administration of potassium iodide for a few days also facilitates expectoration.

Loeffler's Modified Antiformin Method. (Antiformin, a mixture of equal parts of a solution of chlorinated soda and of a 15% solution of caustic soda, can be used to isolate the bacillus from the sputum). To 5 to 20 ml. of sputum add equal volume of Antiformin 50% diluted with water. Heat until clear liquid results. To 10 ml. of the mixture add 10% solution of chloroform in alcohol (5 ml. generally suffices). After shaking, centrifuge 15 minutes. An opaque layer is then formed between the chloroform which occupies the

bottom of the centrifuge and the supernatant fluid. Pipette off the latter and remove the opaque layer wholly on to a slide. Make films, fix and stain. This method is said to be rapid and simple and to give good results.

Alternatively, the sputum is mixed with an equal quantity of a 30% dilution of Antiformin, and the mixture incubated overnight at 37°. After centrifuging, the fluid is poured off and replaced by an equal bulk of normal saline. After shaking up, again centrifuge. Films from the deposit thus washed adhere better to the slide.

Flotation Technique (Ligroin Method). To 5 ml. of sputum in a flask add 50 ml. of caustic potash solution 5%. Shake and leave at room temperature until the sputum is homogenised. Dilute with 50 ml. of tap water and shake again. Add 2 ml. of ligroin and shake until emulsion is formed. Warm to 60° until evidence of layer of smaller bubbles appears on the surface. A number of drops are then taken from immediately below this superficial layer and placed on a warm slide. The dry film is then fixed with saturated sublimate solution and stained by Ziehl-Neelsen method. The ligroin causes the tubercle bacilli to rise to the surface of the meeting of the two liquids.

Higher concentration and better distribution of the bacilli, clearer smears and hence a greater likelihood of detecting the organisms are claimed as advantages of the flotation technique. Xylol is used to separate the bacilli from an emulsion of sputum with 0.5% NaOH, shaken and incubated. The xylol containing the bacilli is subsequently broken down with alcohol or acetone and centrifuged, the supernatant fluid being used for making smears which are stained with carbol-fuchsin allowing rather longer time than usual for the stain to act.—R. L. G. Proctor, *J. R. nav. med. Serv.*, 1941, 2, 149.

Examination of Laryngeal Swabs. Where sputum is not available, an alternative and easier method than examination of gastric contents or fæces is the use of the laryngeal swab. This technique can be used culturally for the isolation of tubercle bacilli from apparently sputum-free patients; the results are particularly good in women, in those undergoing collapse therapy and in the diagnosis of early infiltrative lung lesions. The swab, moistened in water, is passed into the larynx, with or without the aid of a mirror, and the patient is asked to cough. On receipt at the laboratory the swab is placed for 5 minutes in 10% sulphuric acid, transferred to 2% sodium hydroxide for 5 to 8 minutes and then rubbed over the culture medium.—*Lancet*, ii/1941, 317.

Examination of Stomach Contents. Stomach contents obtained by gastric lavage may be examined in the case of adults with little or no sputum or in the case of children who swallow their sputum.

Give 2 grains of potassium iodide three times daily for 2 days before the lavage. Encourage child to cough first thing in the morning for 20 minutes. Then give 100 ml. boiled water to drink. Fifteen minutes later pass stomach tube and withdraw as much fluid as possible. Centrifuge and treat the deposit with 1:10 Antiformin (4 ml. diluted Antiformin being added to 100 ml. lavage).

Examination of the gastric contents for tubercle bacilli by staining direct smears is unreliable. Animal inoculation is more sensitive than cultural methods, but positive results are often obtained when animal inoculation proves negative; both methods should, therefore, be used as a routine. The negative result of a single gastric lavage is not reliable evidence of absence of active pulmonary tuberculosis. (Results of examination of 114 patients).—G. G. Kayne and A. G. Hounslow, *Brit. med. J.*, i/1939, 1173.

Examination of gastric contents is now a recognised valuable aid in the diagnosis of pulmonary tuberculosis in adults with little or no sputum and in children who do not expectorate but swallow it. In the authors' experience every case of tubercle-containing gastric contents gives a positive reaction to tuberculin and the problem is to locate the focus. Primary gastric tuberculosis is rare enough to be negligible and tonsillar tuberculosis would probably be evidenced by enlarged cervical glands so that in the great majority the site is an unhealed pulmonary lesion or a gland ulcerating into a bronchus, bacilli being coughed up and swallowed.—C. Floyd and E. Friedman, *Amer. Rev. Tuberc.*, 1941, 43, 438.

Stomach lavage will find tubercle bacilli that became free in the larger bronchii in a manner not equalled by any other method used at present, and it should be adopted as the ultimate standard for absolute negativity or apparent cure of patients having had or suspected of having tuberculosis. It is an excellent

method to control the efficiency of collapse therapy. No patient with clinical signs of tuberculosis who has negative sputum should be considered negative until gastric aspiration has yielded negative results.—A. Stadnichenko, *J. Amer. med. As.*, i/1940, 634.

Examination of Urine, Pleural Fluid and Peritoneal Fluid. Centrifuge and make films from the deposit. The deposit from a twenty-four hours' specimen of urine should be used and treated with Antiformin. After the film has been stained and decolorised with acid, it must be treated with alcohol for 2 minutes to exclude smegma bacilli.

Examination of Cerebrospinal Fluid. Stand in a sterile stoppered tube for an hour after withdrawal. In tuberculous fluid a "spider-web" coagulum usually forms. This should be removed with a sterile wire loop and placed on a slide; put on cover-slip and press down firmly; remove cover-slip and stain the film. The C.S.F. may be centrifuged and a film made from the deposit.

Examination of Fæces. Formerly it was thought that the discovery of *M. tuberculosis* in fæces was diagnostic of tuberculous enteritis—the bacillus, however, frequently occurs in fæces of patients suffering from pulmonary tuberculosis.

A small piece of fæces (about a cubic $\frac{1}{2}$ inch in size) is placed in a conical glass and to this some 20 ml. of Antiformin diluted with water to 15% is added and the whole well mixed. More of the diluted Antiformin is added and the mixture allowed to stand for about an hour. A white curdy precipitate appears on mixing and settles. Beneath this white layer some unchanged faecal matter remains and above the white layer the fluid is of a clear yellow or brownish colour. A drop or two from the white curdy layer is mixed with a drop of albumin water and stained by the Ziehl-Neelsen method. Much searching may be necessary. For certainty, alcohol may be used in addition to acid for decolorising.

In cases where the sputum is repeatedly negative for tubercle bacilli or where it is not possible to obtain a specimen of the sputum, the fæces should be examined for acid and alcohol-fast bacilli. In a series of 2276 examinations of fæces from cases in which there was no sputum, or in which it was repeatedly negative, 78, or 3·4%, showed these bacilli. The patients had all been in residence at the hospital for at least a week, only pasteurised milk had been consumed and there were no signs or symptoms suggesting tuberculosis of the digestive tract.—G. B. Dixon, per *Rep. med. Offr Minist. Hlth, Lond.*, 1936, 87.

Staining Methods

To exclude acid-fast bacilli and all other bacteria except tubercle and leprosy, wash film in alcohol after fixing by radiant heat. Stain with hot carbol-fuchsin. Differentiate in 25% sulphuric acid and wash freely in tap water and alcohol. Counterstain in picric acid and alcoholic solution. Dry and examine by $1/12$ inch immersion lens.

Ziehl-Neelsen Method. 1. Prepare film from sputum or a section ready for staining, and fix by usual methods. 2. Heat filtered carbol-fuchsin in a test-tube and cover specimens with it entirely; stain films 5 minutes, sections 10 minutes. (**Carbol-Fuchsin Solution**, Neelsen's solution, is prepared by mixing concentrated alcoholic fuchsin solution 1 with 5% phenol solution 9, slightly warmed.) 3. Wash well in water. 4. Decolorise almost completely by immersing in 25% sulphuric acid. (If 3% hydrochloric acid in 95% alcohol be used instead, smegma and similar organisms are excluded). 5. Wash well in water. 6. Counterstain with alkaline methylene blue or carbolised methylene blue. 7. Wash, dry and mount in xylol balsam (sputum). 8. If section, dehydrate with alcohol, clarify with xylol and mount in xylol balsam. If dehydrated with aniline instead of alcohol a clearer preparation is produced.

Alkaline methylene blue is prepared by mixing saturated alcoholic (medicinal) methylene blue solution, 142 m., with 1 oz. of a 1 in 10,000 solution of caustic potash. **Carbolised methylene blue** is prepared by dissolving methylene blue 1 as much as possible in alcohol 90% 7, adding phenol solution 5% 70, allowing to settle and decanting.

Rapid Staining Method. Spread material on slide, apply Ziehl's carbol-fuchsin, heat slide till it steams, and wash in running water. Then place for 40 to 50 seconds in a solution of: brilliant yellow 0·15 g., concentrated sulphuric

acid 10 ml., alcohol 20 ml., and distilled water 85 ml. Again wash and dry with blotting paper. The bacilli are coloured red on a lemon-yellow background. Only takes half the time of Ziehl's method, the bacilli are clearer and more numerous, and it is just as reliable as the Ziehl-Neelsen test.

Spengler's Method. (1) Stain with carbol-fuchsin, steaming for 3 to 5 minutes, (2) pour off carbol-fuchsin and apply picric acid alcohol (2 g. picric acid in 40 ml. distilled water; stand for 24 hours, filter and add equal vol. 96% alcohol) for 2 to 3 seconds, (3) apply 3 to 4 drops of 15% nitric acid for 5 seconds, (4) pour off and apply picric acid alcohol till sputum looks yellow, (5) wash, dry and mount. Considered by many superior to all other methods.

Rosolic Acid Method. Specially for *M. tuberculosis* in tissues. Stain in hot carbol-fuchsin for 5 minutes. Wash quickly in tap water. Dip five or six times in saturated alcoholic solution of rosolic acid (corallin) till fuchsin is removed. Wash in water and counterstain in saturated alcoholic solution of methylene blue.

Konrich's Method. Stain with hot carbol-fuchsin for $\frac{1}{2}$ to 2 minutes, rinse with water, decolorise with 10% sodium sulphite solution for $\frac{1}{2}$ to 1 minute, rinse with water, then counterstain with malachite green (50 ml. of saturated aqueous solution of malachite green in 100 ml. distilled water) for $\frac{1}{2}$ to 1 minute.

Fuchsin-Aniline Green Method. Solution A. Fuchsin 10, absolute alcohol 100. Solution B. Strong ammonia solution 3, water 100. Solution C. Water 80, nitric acid 20, malachite or iodine or acid green *q.s.* to saturate. Methyl green does not give satisfactory results.

Add one part of A to 10 of B. Warm until vapour arises, immerse 1 minute, wash with water, then immerse in C 40 seconds. Wash off thoroughly. Bacilli red on pale green ground.

Indirect Methods of Diagnosis. The most important of these depend on the presence in the tissues of sensitising and immunising substances. They are indicated in doubtful cases of tuberculosis in which no material is available for investigation by the direct methods outlined above, or they may be employed in addition to these methods.

(1) Complement-fixation Test. This may be applied in the diagnosis of some cases of tuberculosis and also as an index of the activity of the disease. There is no direct relationship between the degree of hypersensitiveness and the gravity of the disease. It is most often positive in chronic but active lesions; in arrested cases and also in rapidly progressive tuberculous conditions it may be negative.

Preparation of the Antigen. Use a young, rapidly-growing culture on a good medium (e.g., Dorset's egg). Inoculate 6 tubes, allow to grow for 10 to 14 days, scrape off growth, and prepare emulsion in the proportion of 1 g. of the bacilli to 50 ml. carbol-saline (0.25%); store the antigen in a refrigerator. As a rule 0.05 ml. to 0.1 ml. is required in the test.

The Test. (1) *Estimation of the minimal hæmolytic dose (M.H.D.) of the complement.* Place falling doses—from 0.35 ml. to 0.05 ml.—of the complement, diluted 1 in 24 with saline, in a series of test tubes: make the total volume up to 1 ml. with normal saline, and add 0.5 ml. of a 5% suspension of sensitised sheep's corpuscles (6 units of amboceptor), incubate tubes in water-bath for 10 to 20 minutes and note the tube with the least amount of complement showing hæmolysis; two and a half times this amount is the complement required in the standardisation of the complement and in the actual test. (2) *Standardisation of the antigen.* Place falling doses of antigen—0.2 ml. to 0.025 ml., in a series of 6 tubes, adding 2½ units (M.H.D.) of complement diluted to give a volume of 0.5 ml. to each, and add saline up to 1 ml.; shake tubes and incubate at 37° for one hour; add 0.5 ml. of 5% suspension of sensitised sheep's corpuscles; shake tubes and place in water-bath for 10 to 20 minutes. The tube containing the largest quantity of antigen (usually 0.1 ml.) showing complete or nearly complete hæmolysis is the amount of antigen to be used in the test. (3) In the test, 0.1 ml. of patient's serum and of a control normal serum is used and five test-tubes filled as follows:—

| Tube | Patient's Serum | | Control Serum | | Antigen Control V |
|-------------|-----------------|---------|---------------|---------|-------------------------|
| | I | II | III | IV | |
| Antigen ... | 0.1 ml. | — | 0.1 ml. | — | 0.1 ml. |
| Serum ... | 0.1 | 0.1 ml. | 0.1 | 0.1 ml. | — |
| Complement | 0.25 " | 0.25 " | 0.25 " | 0.25 " | 0.25 " |
| Saline ... | 0.55 " | 0.65 " | 0.55 " | 0.65 " | 0.65 " |

After mixing, incubate tubes for one hour, then add 0.5 ml. of the sensitised red cells to each, shake, place in water-bath for 10 minutes and note occurrence of hæmolysis. Tubes II, III, IV and V should show complete hæmolysis and also tube I if the patient's serum is negative, but if positive there will be little or no hæmolysis in I.

(2) **Tuberculin Tests.** These depend on the fact that infection by the tubercle bacillus renders the tissues hypersensitive to its toxins if these are introduced artificially, giving rise to the production of reactions which are regarded as more or less specific. Of the various tests employed the best known are the subcutaneous test (Koch), the cutaneous test (Pirquet), the intradermal test (Mantoux), the percutaneous test (Moro) and the patch test (Vollmer). The two most frequently employed now are the intradermal test and the patch tests. (For details of all these tests, see Vol. I, pp. 1084-1087.)

Old Tuberculin. Standard, B.P. The standard preparation is a quantity of old tuberculin kept in the National Institute for Medical Research, Hampstead. There is no unit of activity for old tuberculin.

Method of Comparison. To compare unknown samples with the standard, guinea-pigs are sensitised by injecting them intramuscularly with 0.25 mg. to 0.5 mg. of living bacilli from a 3 weeks' growth of *M. tuberculosis*. Sensitisation follows in 3 weeks. Injections are then made into the shaven skin of the flanks of the guinea-pig. On one side dilutions of the standard are injected, the dilutions being 1 in 1000, 1 in 2000, 1 in 4000. On the other side similar dilutions of the unknown are injected. Twenty-four hours later the sites of injection are examined to compare the inflammatory reactions. Those produced by the unknown sample should be indistinguishable from those produced by the standard.

(3) **Guinea-pig Inoculation.** This method may be used to demonstrate the organisms when they cannot be found by other methods and to distinguish between tubercle bacilli and non-pathogenic acid-fast bacilli. The animal is injected subcutaneously in the groin (if the material is badly contaminated it must be treated with acid or alkali, as in making cultures, *q.v.*). If the tubercle bacillus is present, a local swelling develops which may caseate and ulcerate. Smears from the ulcerated area often show the bacilli. The regional glands become involved and later the mesenteric and other lymph nodes. The lesions may be looked for in from 4 to 6 weeks and death usually occurs in about 2 months. At autopsy the characteristic lesion is an enormous enlargement of the spleen, which is studded with grayish or yellowish tubercles. Infected guinea-pigs become highly sensitive to tuberculin after 2 or 3 weeks and will die within 24 hours if given a subcutaneous injection of 1 or 2 ml. of old tuberculin at this time.—Stitt.

(4) **Cytological Test.** Very useful evidence of tuberculous infection is to be obtained in cases of pleural, peritoneal and meningeal exudates by estimating the relative numbers of polymorphonuclear cells and of lymphocytes. It is found that in pure tuberculous infection the cell exudate is largely, and often almost entirely, lymphocytic in character. In pyogenic infections it is very often largely polymorphonuclear; in mixed infections (tubercle with pyogenic infection) the cell exudate is also of a mixed character.—*Price's Textbook of Medicine*, 6th Edn., 1941.

(5) **Sedimentation Rate.** Valuable evidence as to the progress of the disease and the value of treatment may be obtained by a study of the blood sedimentation rate. (For further details, see this volume, p. 682.)

CULTIVATION

Primary growths of *M. tuberculosis* can be obtained on serum-media or on one containing egg-yolk (see p. 976); secondary cultures may be made on agar, broth

or potato media containing 5 to 6% glycerol. The bacillus requires a temperature of 37° and produces a dry wrinkled growth, somewhat like a lichen, on glycerin agar in three weeks. Cultures, especially in glycerinated broth, have a fruity odour.

A slow, but certain method to obtain a pure culture of the organism from tubercular material is to inoculate guinea-pigs with same, and after a lapse of four to six weeks, cultures are made from enlarged glands if these have not ulcerated through the skin.

Petroff's Method (Modified). Any volume of sputum up to a maximum of about 5 ml. is thoroughly mixed with three or four times its volume of 4% caustic soda solution. The mixture is incubated at 37° for 30 minutes and centrifuged for one hour at 3000 r.p.m. The supernatant liquor is decanted and the deposit rendered neutral to phenol-red with 8% hydrochloric acid. The deposit is then thoroughly mixed with a glass rod and thickly sown on to Lowenstein-Jensen's medium (*q.v.*) contained in screw-capped bottles. Incubate at 37°. Growth is usually visible in twenty-one days, but even when no colonies are visible at the expiration of this period, there may be a good growth of *M. tuberculosis* as an extremely thin film on the surface of the medium. No cultures should, therefore, be discarded as negative until they have been examined microscopically since transplants from these film growths grow vigorously and typically. 6% sulphuric acid may be used in place of hydrochloric acid and neutralisation effected with NaOH. Cultures may be made on Petroff's medium (egg medium containing 1 : 10,000 crystal violet).

Lowenstein-Jensen Medium. Peptone is not conducive to the growth of *M. tuberculosis* and in this medium is replaced by asparagin. A solution of potassium dihydrogen phosphate (KH_2PO_4) 0.4%, magnesium sulphate 0.04%, magnesium citrate 0.1%, asparagin 0.6% and glycerol 2% in distilled water is heated in the steamer for 2 hours and cooled. To each 600 ml. of the solution are added 30 g. potato starch and the mixture heated on a water-bath, with stirring, for 15 to 20 minutes to form a paste; it is then kept for 1 hour in a water-bath at 56°, after which is added 1 litre of egg fluid prepared as follows:—

Hen's eggs, less than a week old (previously washed in 5% soft soap solution to disinfect them, and in running water for 1 to 2 hours) are broken into a sterile vessel and the white and yolk mixed thoroughly and filtered through sterile gauze. To this mixture is added 20 ml. of 2% malachite green solution in distilled water which has been incubated for 1 or 2 hours. Quantities of 5 ml. are placed in 1 oz. screw-capped bottles which are laid horizontally (after the caps have been tightly screwed on) and heated in the inspissator at 75° for half an hour. They are kept at 37° overnight and again heated to 75° for half an hour on the following day.

This medium shows good primary growth of *M. tuberculosis* from sputum treated with 4% NaOH in ten to twelve days. The human type of the organism grows very luxuriantly as large heaped-up colonies which are yellow; the bovine type grows as small discrete colonies which are colourless.

Long's Synthetic Medium (for preparation of Tuberculin P.P.D.—see Vol. I, p. 1079). Glycerol 50 ml., asparagin 5 g., sodium citrate 6 g., KH_2PO_4 2 g., ammonium chloride 1 g., magnesium sulphate 0.5 g., ferrous ammonium citrate 0.05 g., tap water 1 litre.

Rapid Culture Method. This consists in drying a film of sputum on a glass surface, treating with 15% sulphuric acid, washing with water and incubating with blood hemolysed with distilled water or 1% saponin. After seven days' incubation the preparation is washed and stained with Ziehl-Neelsen and the colonies examined with the low power. The most typical colonies are long and stringy, later becoming twisted and coiled, but compact and bushy growths occur. Mycelial threads are found and it is possible that growth does not take place throughout the length of the thread but is restricted to certain points. Obvious signs of growth are visible with the $\frac{1}{2}$ in. objective in 24 hours, colonies may be just perceptible with the $\frac{1}{2}$ in. objective in 48 hours, and in three days they are usually quite distinct. There are two methods of applying this dried-film culture technique. In one the sputum is spread on the floor of a petri dish. In the other, one or more patches of sputum are spread on a glass slide, dried, surrounded with a ring of bakelite, hard fibre or glass immersed in melted soft paraffin, and incubated in a moist chamber with a cover-slip to cover the ring.—D. M. Pryc *J. Path. Bact.*, 1941, 53, 327.

Relationship Between Human and Other Forms of Tuberculosis

Different types of *Mycobacterium tuberculosis* are differentiated as "human," "bovine" and "avian," occurring in man, cattle and birds respectively.

In morphology and staining reactions the bovine and avian types are practically identical with the human type. The bovine type shows less luxuriant growth than the human type on culture media and is described as "dysgonic." On egg media the cultures are smooth, white, granular, slightly moist and easily broken up. (The human type is "eugonic" = good-growth.) Glycerol favours the growth of the bovine bacillus and the difference between the two types is accentuated by the use of a glycerol-egg medium. Besides its greater virulence for cattle, the bovine type is more virulent than the human type for laboratory animals. This can be demonstrated by injecting an emulsion of 0.01 to 0.1 mg. of dried tubercle culture intravenously into rabbits. The human type produces slight lesions mainly confined to the lungs and kidneys and the animals survive for more than two months. The bovine type produces acute generalised tuberculosis from which the animals die within two months. If the injections are made subcutaneously (dose 10 mg.) into rabbits, the human type produces only a local lesion whereas the bovine type produces a fatal generalised tuberculosis. The avian type grows best at 41° to 42° and cultures on glycerol-agar develop more rapidly, are wetter and more homogeneous than the human or bovine types; cultures are more luxuriant and the colonies are large, hemispherical, glistening and yellowish in colour. Highly virulent for fowls but of very low virulence for guinea-pigs.

The bovine bacillus is at least as virulent for man as the human bacillus, as shown by its incidence in cases of tuberculous meningitis in Great Britain. In children under 15 years 26.5% of cases in England and 28.3% in Scotland were due to bovine infection; in patients over 15 years of age the figures were 16.6% and 26.2%. Bovine strains isolated from pulmonary tuberculosis in England account for only 1.6%, and only 1% in Wales, but in Scotland they occurred in 7%. In human cutaneous tuberculosis the bovine and human types are responsible for infection in equal proportion.—A. S. Griffith, *Tubercle*, 1941, 33; *Lancet*, i/1941, 637.

At Glenlomond Sanatorium bovine organisms have been isolated from 20 males and 38 females suffering from pulmonary tuberculosis. In comparison with a series of 20 males and 38 females whose disease was due to the human bacillus, a higher proportion of bovine strains was evident in the age group 10–20 years, especially among females. The bovine type cases showed a high mortality rate; of the 38 female cases 22 were dead, 11 ill, and only 5 well; of the 20 males, 12 were dead, 5 ill, and 3 well. Gastric lavage applied to infected children showed 14 bovine and 21 human strains and the bovine type was more easily disseminated with the production of multiple lesions and tuberculous bacilluria. Munro is convinced that the bovine strain is more virulent to man than the human strain. Once the bovine bacillus passes the lymphatic barrier it is easily disseminated, the onset of serious visceral spread in the lungs and kidneys occurs at an earlier age and the whole duration of the illness is shorter. There are three possible ways in which infection can be spread: man to man; cattle to man in the course of dairy work; and cattle to man through infected milk.—W. T. Munro, *Edinb. med. J.*, 1940, 47, 110.

For further details re bovine tuberculosis and milk infection see *Milk Analysis* this volume.

Tuberculosis in dogs is comparatively rare—it is almost invariably due to infection from a human source. The symptoms—emaciation, loss of strength, etc., are easily recognised. On the other hand, the prevalent opinion that dogs are practically immune to tuberculosis is erroneous. In 3 years 165 cases were recorded, all being verified anatomically and bacteriologically. The disease is more prevalent among dogs in town than in country districts. Cats also are capable of infection, but are less frequently affected than dogs. Horses seem to be very rarely affected, scarcely one in 15,000 cases examined has been recorded.

Voles are highly susceptible to the bovine type of tubercle bacillus whereas they are resistant to the human type. Tuberculosis occurs under natural conditions in the vole and is not uncommon in the mink and silver fox (see also *Vol. I*, p. 1084).

Tularæmia. *Syn.* RABBIT FEVER, DEER-FLY FEVER, PAHVENT VALLEY FEVER, OHARA'S DISEASE. A plague-like disease, caused by

Pasteurella tularensis (*Bacterium tularense*), which affects animals and men. In animals it has the virulence of plague, but in man the symptoms are much milder. The disease was first noticed in 1911 among ground squirrels in Tulare County, California. Subsequently the organism was found in various rodents, and cases of tularemia have been reported from all parts of the United States and from Japan, Canada, Russia, Norway, Sweden, Czechoslovakia, Austria, Turkey and Asia Minor. The incidence of the disease is highest among those who are brought into contact with infected animals, e.g., butchers, farmers, trappers, hunters, and cooks.

Four different clinical types of the disease have been described. (1) *Ulceroglandular type*. In this type there is a necrotic papule at the site of inoculation, with enlargement, pain, and sometimes suppuration of the lymph nodes related to the area of infection. There is considerable pyrexia and toxæmia. (2) *Oculoglandular type*. Conjunctivitis with enlargement of regional lymph nodes (and, in very severe cases, of the axillary group). (3) *Glandular type*. There is an absence of primary lesions but there is enlargement of lymph nodes (especially epitrochlear and axillary). (4) *Typhoid type*. There is an absence of primary lesions and no glandular enlargement. Fever is the only outstanding symptom. This type is particularly common among laboratory workers.

The disease is not fatal, unless complications such as bronchopneumonia set in, but it causes a long period of inability to work and convalescence sometimes lasts as long as six months. An attack of the disease is stated to confer a lasting immunity.

Thirty cases of tularemia reported in Sweden in 1931; most were caused by insect bites. Fifty-one cases diagnosed between August and October, 1934; seven of these showed co-agglutination with *Br. abortus*. In only one instance could the disease be traced to direct contact with rodents.—B. Malmgren, *Bull. Off. int. Hyg. publ.*, 1935, 27, 2184.

Two fatal cases of tularemia contracted by eating uncooked or partly cooked rabbit.—A. H. Amoss and D. H. Sprunt, *J. Amer. med. Ass.*, i/1936, 1078.

Three fatal cases of tularemia which were traced to the dressing of wild rabbits occurred amongst 23 cases of tularemia in Cleveland. The clinical course was characterised by hyperpyrexia, severe toxicity, delirium and coma. Treatment by sulphanilamide (one case), specific immune serum and convalescent serum was not effective. *Pasteurella tularensis* was not found in the circulating blood but was recovered at necropsy from lymph nodes in two cases, and from the hæmorrhagic mass in the rectus muscles in the third. Bacterial stains did not reveal the organisms in tissue sections. The lesions in lymph nodes, spleen, liver and lungs were characteristically those of a necrotising inflammation in which large mononuclear cells, often actively phagocytic, predominated.—L. L. Terry and H. S. Reichel, *Arch. Path.*, 1940, 29, 473.

Morphology. *P. tularensis* is a small, non-motile, Gram-negative bacillus, $0.3\ \mu$ in length and $0.2\ \mu$ in breadth. It is not easily stained by the ordinary dyes unless a mordant such as phenol or aniline is added. It is refractory to growth on ordinary media and will grow only on coagulated hen's egg-yolk or on blood-glucose-cystine-agar. It is readily killed by heat and causes fermentation with acid production in dextrose, glycerin, mannose, maltose, dextrin and lævulose.

Transmission. The disease is transmitted to man from rodents, not by direct human infection. Wild rabbits cause more than 90% of the cases; domestic rabbits have not been found naturally infected. The disease is usually acquired by handling the tissues of infected animals, the bacteria gaining entrance either through the skin or through the conjunctive. It may also be acquired by the bite of the horse-fly (*Chrysops discalis*) or of ticks (such as *Dermacentor*

andersoni or *Dermacentor variabilis*), or from the bites or scratches of animals which have eaten infected material. Cases have also been reported following the ingestion of insufficiently cooked rabbit meat or of infected water.

Polluted water has been regarded as an important source of infection. In Norway it is thought that "lemming fever" which follows the drinking of water polluted with the bodies and excreta of animals is a form of tularæmia. In America it has now been found that the water in three Montana streams is contaminated with *Bact. tularensis*. The source of this infection was found to be infected beavers (*Publ. Hlth Rep., Wash., 1940, 55, 227*).—P. Manson-Bahr, *Med. Annu.*, 1941, 399.

Diagnosis. Direct cultivation is difficult and unpractical for diagnosis. The most satisfactory method is animal inoculation. Material from the primary lesion, regional lymph nodes, or blood is injected subcutaneously or intraperitoneally into guinea-pigs, rabbits or mice, which usually die within a week. At autopsy the animal shows a hæmorrhagic œdema at the site of inoculation, caseation of the lymph nodes, and small necrotic foci in the spleen and liver. Smears from these tissues stained with crystal violet usually show numerous organisms and they may be cultivated from the lesions and from the heart's blood. Blood-glucose-cystine-agar is inoculated with the blood or with small pieces of liver and spleen. Growth appears about the third day.

Agglutination tests are a valuable means of diagnosis after the first week, a titre of 1:80 or over being considered diagnostic. (Agglutinins develop in guinea-pigs 10 to 14 days after they have been infected by cutaneous inoculation).—Stitt.

Blood-Glucose-Cystine-Agar. This medium is made as follows: Fresh beef infusion containing 1% peptone, 1 or 1.5% agar and 0.5% NaCl, and adjusted to pH 7.3, is kept in stock. When needed there is added to the stock agar, 0.1% cystine and 1% of glucose. This is placed at the temperature of flowing steam in a steriliser sufficiently long to melt the agar and sterilise the cystine. Cool to 60° and add 5 to 8% of defibrinated or whole rabbit blood. Sterilise by heating the flask of blood-glucose-cystine-agar in a water-bath for 2 hours at 60°.

Typhoid. Characters of *Bacillus typhosus* (syn. BACTERIUM TYPHOSUM, EBERTHELLA TYPHOSA). *B. typhosus* is a motile rod 4 μ long, but its length varies on cultivation; its motility is due to flagella, which are 12 to 16 in number. It is Gram-negative and grows easily in ordinary media. It produces acid without gas in dextrose, maltose, lævulose, galactose and mannitol. It causes an acidity in milk, but does not form a clot. It does not form indole in peptone water, nor liquefy gelatin.

B. typhosus will not grow below 15° or above 41°; it is killed in 15 minutes at 60° and practically instantaneously at 100°. It may retain its infectivity in ice for months and can survive for 2–3 weeks in water. It is very susceptible to acidity. In wine it rapidly disappears, while wine added to water will reduce the number of organisms if present; 20 ml. of vinegar per litre kills *B. typhosus* in one hour.

Antigenic Structure of *B. typhosus*. The typhoid bacillus contains characteristic somatic or "O" antigens associated with the body of the bacillus, and flagellar or "H" antigens associated with the flagella. These two antigens produce different agglutinins, also designated by the letters "H" and "O", and the agglutination which results from the interaction of specific antigens and agglutinins is denoted by the same symbols. H-agglutination is of the large-flake type in which the clumps are easily visible; O-agglutination is of the small-flake or granular type. Differential testing for H and O agglutinins is an important procedure in the

identification of pathogenic intestinal bacteria (*see* Widal Reaction and Salmonella Group of Organisms).

Recently isolated strains of *B. typhosus*, which are more virulent for mice than ordinary stock cultures, possess an additional somatic antigen which has been designated "Vi" because of its association with virulence. The presence of the Vi antigen renders the organism inagglutinable by O antiserum. The Vi antigen gradually disappears on repeated sub-culture and the strains lose their virulence and become agglutinable by O antiserum. The Vi antigen is also destroyed by heating for 10 minutes at 100° and is partially destroyed by phenol. It is best developed when the organism is grown on soft ascitic-fluid-agar medium. (*For details of specific Vi antiserum, see Vol. I, p. 1089.*)

Diagnosis. A typical case of enteric fever can be diagnosed without difficulty, on clinical grounds alone, at any time after its first week. Except in epidemics, however, it is only too common for such cases to be missed until a fatal issue, or the occurrence of other cases, arouses suspicion. Any fever lasting for more than three days with no obvious explanation demands the help of the laboratory to exclude or confirm an enteric infection. Culture and identification of the specific bacteria in the blood or excreta are practicable in the earliest stage of the disease; they are most successful at the onset and more difficult later and are the first laboratory tests to be applied. Culture in the late stages and in convalescence, when blood and excreta are both negative, can sometimes be made from the bone marrow (sternal puncture), but after the second or third week the Widal test is the chief diagnostic aid. Marr's test is of value where laboratory aid is not available and is useful between the fifth and fourteenth days.

Marris's Atropine Test. Atropine 1/33 grain hypodermically barely increases the pulse rate in typhoid and paratyphoid "A" and "B" infections, whilst in normal people and those suffering from other diseases it accelerates it. At least one hour should elapse after a meal. Give the injection and allow 25 minutes to elapse—patient remaining absolutely quiet before making second observation. As an arbitrary rule an increase of pulse rate by about 20 or more beats a minute after the injection may be accepted as an indication that patient is probably *not* suffering from typhoid or one of the paratyphoid series. If the increase is only 10 beats or less the reaction is suggestive of infection.

CULTIVATION

Blood Cultures. Blood for culture should be drawn from a vein as soon as suspicion of enteric arises. An anti-coagulant (citrate or sterile ox-bile) should preferably be present in the receptacle. Ordinary clotted blood, though a little more troublesome to manipulate, is probably at least equally good for culture purposes and provides in addition, serum which may be useful later for comparison with later tests (even when it gives negative agglutination tests as it may in the early stages of the disease).

Conradi evolved a method of early diagnosis of typhoid fever. Researches demonstrated necessity of keeping the blood in a fluid condition, so as to avoid the disinfectant action of those substances which become active on coagulation. Bile is employed for this purpose; in addition, the medium contains 10% of peptone and 10% of glycerin. The blood from lobe of the ear is drawn into a pipette containing a little bile and mixed with 2 ml. or 3 ml. of the peptone-glycerin-bile medium in the proportion: blood 1, medium 3. Incubate at 37° for 10 to 16 hours and make cultures on agar plates according to the

Drigalski-Conradi formula. Diagnosis can be effected by this method in 26 to 32 hours and it is applicable as soon as the patient exhibits a febrile temperature.

Drigalski-Conradi Medium consists of a nutrose-lactose-litmus agar containing 1% nutrose (a sodium caseinate compound), 1% peptone, 0.5% salt, 3% agar, 1.5% lactose, in a nutrient broth made with 750 g. horse flesh to the litre, also 13% of Kubel and Tiemann's litmus solution and a trace (0.001%) of crystal violet. After incubation typhoid colonies are blue, glassy like dew drops, paratyphoid are similar, and *B. coli* are bright red and opaque.

Faecal Cultures. Faecal specimens should be from stools freshly voided into a receptacle which has been recently scalded out with boiling water, about half a teaspoonful being transferred into a sterile screw-cap bottle. If delay of more than 18 hours is likely before the specimen can reach the laboratory, the bottle should contain 5-10 ml. of sterile glycerin-saline (30% neutral glycerin in 0.6% saline). If a fresh specimen of faeces is not available, a rectal swab may be taken instead, using an ordinary throat swab for the purpose.

For the cultivation of *B. typhosus* from faeces, the medium which has been most generally used is **MacConkey's bile-salt-neutral-red-lactose-agar** (see p. 860). On this, the colonies of *B. typhosus* are pale in contrast to the pink colonies of *B. coli*. It is essential that the surface of the medium should be dry before it is inoculated, since even a small amount of condensation water will cause a confluent growth instead of discrete colonies.

Endo's Medium (as modified by Robinson and Rettger). Agar 25 g., beef extract 3 g., peptone 10 g., distilled water 1 litre. Dissolve the agar in 900 ml. of water in autoclave at 15 lbs. for 40 minutes. Dissolve peptone and meat extract in 100 ml. water in water-bath. Mix. Make up volume to 1 litre. Adjust to pH 8.5 with 10% sodium carbonate. Filter while hot and sterilise in autoclave.

To use, melt, and to each 100 ml. add 10 ml. of 10% lactose solution and 0.26 ml. saturated alcoholic solution of basic fuchsin and 1 ml. of 10% solution sodium bisulphite. Colonies of *B. typhosus* are greyish white—those of *B. coli* are red.—Mackie and McCartney.

Various methods are used to inhibit non-pathogenic intestinal organisms and facilitate the isolation of *B. typhosus* and *B. paratyphosus*:—

Wilson and Blair's Bismuth Sulphite Medium. (A) Dissolve 30 g. of bismuth ammonium citrate scales in 250 ml. of distilled water. Add a boiling solution of 100 g. anhydrous sodium sulphite in 500 ml. of distilled water and while the mixture is still boiling add 100 g. of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$. Cool and add solution of 50 g. of glucose in 250 ml. of distilled water. (B) 1% solution of ferric citrate scales in distilled water 200 ml., 1% brilliant green in distilled water 25 ml. These solutions may be kept for months.

The medium is prepared as follows:—Nutrient agar (3%), melted and cooled to 60°, 100 ml.; Solution A 20 ml.; Solution B 4.5 ml. It should be poured into petri dishes.

The use of this medium depends upon the property of *B. typhosus* to reduce sulphite to sulphide in presence of glucose and the inhibition of *B. coli* by brilliant green and by bismuth sulphite in the presence of excess sodium sulphite. Isolated colonies of *B. typhosus* and *B. paratyphosus* are black. The former appear within 24 hours and the latter within 48 hours.—W. J. Wilson, *J. Hyg., Camb.*, 1938, 38, 507; see also Mackie and McCartney.

Brilliant Green Enrichment Method. In fluid media containing certain concentrations of this dye, *B. coli* is partly or completely inhibited, whilst *B. typhosus* and *B. paratyphosus* grow well. Use three tubes of 10 ml. of peptone water (2% peptone, 0.5% NaCl, pH 7.0), inoculate with liquid stool or dense suspension of solid stool. To the tubes add 1:10,000 aqueous solution of brilliant green in the following amounts:—Tube 1, 0.25 ml.; Tube 2, 0.4 ml.; Tube 3, 0.7 ml. Incubate at 37° and at end of 12 to 18 hours sub-culture on MacConkey's medium. This method is even more satisfactory for *B. paratyphosus* than for *B. typhosus*. Under favourable conditions almost pure growths of the enteric organism may be obtained. (For composition of **Brilliant Green Agar Medium**, see p. 998.)

Tetrathionate Medium (see under *B. paratyphosus*, p. 984).

Urine Cultures. Urine specimens voided into a sterile receptacle should be transferred in 2-4 oz. samples to sterile bottles for transmission. The urine should be centrifuged and the sediment cultured or broth flasks heavily inoculated. No patient should be released from isolation until cultures from the urine and faeces, after catharsis, are negative.

STAINING METHODS FOR FLAGELLA

Of the various methods for staining the flagella of bacteria that have been devised, those described by J. Kirkpatrick, Fleming and Pitfield (as modified by Muir and Ritchie) are the most generally used. Strict attention must be paid to the cleanliness of the slide used for spreading the film; a preliminary cleansing in dichromate-nitric acid mixture is essential (nitric acid 6, potassium dichromate 6, water 100).

Kirkpatrick's Method. (A) *Fixing Solution*: Absolute alcohol 60 ml., chloroform 30 ml., formalin 10 ml. (B) *Mordant*: 5% ferric chloride solution 1, 20% tannic acid (dissolved by heat and cooled) 3. Dilute with an equal volume of water before use. (C) *Silver Solution*: Silver sulphate 10 g. added to 200 ml. distilled water in a screw-capped, clean, clear-glass bottle; incubate for 24 hours at 37° shaking occasionally. This keeps well and improves on exposure to daylight. Filter 40 ml. of this solution into a clean 100 ml. flask and add rapidly 0.6 ml. of "33% w/v" ethylamine. The precipitate which forms at first redissolves immediately; add from a pipette more of the silver solution until a permanent opalescence remains; finally add 10 ml. distilled water.

To stain flagella use 36-48 hour agar cultures of colityphoid group; for vibrios 24 hour cultures may be preferable. A bacterial suspension is made by carefully mixing a loopful of culture with 1 ml. of water in a test-tube (previously sterilised by flaming) and diluting with sterile distilled water to a dilution of about 200 organisms per ml. A film is spread on a clean slide from a loopful of this suspension (the excess is drawn to one side to act as a control in the staining process) and dried in air. The slide is placed in a jar containing the fixing solution for 1-3 minutes, rinsed in alcohol and washed in water; placed in jar of mordant for 3-5 minutes and well washed in water. The silver solution is filtered on to the slide which is heated by flaming the under side for about 15 seconds (until the control spot is dark brown in colour and a metallic scum appears at the edge of the liquid). Allow solution to act for 15-30 seconds longer without further heating, wash off in running water, dry and mount in balsam. Organisms are stained black—flagella are clearly defined and light brown or grey in colour.

Fleming's Method uses organisms grown on cellophane and avoids transference of culture medium to slide. A disc of cellophane is sterilised in distilled water in the autoclave and placed on the surface of an agar plate which is dried off in the incubator for a short time. The culture is inoculated on to the surface of the cellophane and incubated for not more than 24 hours. The disc is removed with forceps and with the whole of the culture placed in a petri dish with sufficient distilled water to cover the disc; place in incubator for a few hours to allow the bacteria to float off into the water. Add carefully 1 ml. formalin to fix the bacteria and leave undisturbed overnight. The suspension can be poured into screw-capped bottles and keeps well for a long time.

To stain flagella, a drop of a thin suspension is spread over a large part of a slide and air-dried. (A) *Mordant*: 20% tannic acid solution 3 parts, 5% tartar emetic 2 parts and water 5 parts. Heat to boiling to redissolve the precipitate and flood on to slide. After two minutes wash with tap water. (B) *Silver Solution*: Saturated aqueous solution of silver sulphate and distilled water, equal parts; add ethylamine until precipitate just redissolves. Heat almost to boiling and flood on to slide—allow to act for 1 minute, wash off with tap water and dry.

Other methods that have been used are:—

Pitfield's Method. *Solution A*. Tannin 1 g., water 10 ml. Do not filter. *Solution B*. Saturated aqueous solution of alum 10 ml., saturated alcoholic gentian violet solution 1 ml. Filter and keep in a stoppered bottle. Fuchsin will answer the same purpose as gentian violet. Equal parts A and B mixed, heated nearly to boiling and employed to stain 1 to 3 minutes, wash in water, dry and mount.

Muir's Modification. *Solution A (Mordant)*: Tannic acid 10% aqueous solution (filtered) 10 ml., mercuric chloride, saturated solution, 5 ml., alum saturated solution 5 ml., carbol fuchsin 5 ml. Mix thoroughly; separate precipitate by allowing to stand or by centrifuging. Transfer fluid to clean bottle with pipette. Keeps well 1 or 2 weeks. *Solution B (Stain)*: Alum saturated solution 10 ml., gentian violet saturated alcoholic solution 2 ml. The stain should not be more than 2 or 3 days old. Pour over the film as much mordant as slide will hold, heat till steam rises for 1 minute, wash in running water 2 minutes, dry thoroughly over flame, then pour on stain. Heat till steaming for 1 minute, wash in water, dry and mount.

McCrorie's Stains. *Solution A.* Night blue 1 in dehydrated alcohol 20, alum 1 in water 20, tannic acid 1 in water 20. Mix and filter at once. *Solution B.* Aniline fuchsin. To 100 ml. of saturated aniline water, add 10 ml. of dehydrated alcohol and 1 g. of fuchsin, or carbol-fuchsin diluted may be employed.

Van Ermengem's Stains. *Solution A:* 1% Osmic acid solution 100, tannin 18, water 45. *Solution B:* Silver nitrate solution 0.25 to 0.5%. *Solution C:* Gallic acid 1, tannin 0.6, potassium acetate fused 2, water 70.

Plimmer and Paine's Method. Rub down tannin 10, aluminium chloride (cryst.) 18, zinc chloride 10 and rosaniline HCl 1.5 with alcohol 60% 10, then employ a further 30 of the alcohol. In use, the clean slide is baked and allowed to cool to blood heat and a drop of 18 hours' culture placed at one end and allowed to run down by tilting. The film must dry quickly. One part of the stain is mixed with 4 parts of water and after standing 60 seconds it is filtered on to the film and left on for a further 60 seconds, then washed rapidly. Finally stain with carbol-fuchsin 5 minutes, wash and dry.

WIDAL REACTION

In the routine Widal reaction the patient's serum is tested against each of the organisms likely to be responsible for enteric fever in the particular area. It is now customary to test the serum with standard H and O suspensions of each organism. Usually both types of agglutinins are present in the patient's serum, but in some cases—particularly at an early stage—only one of these agglutinins is present. The reaction is usually demonstrable about the seventh to tenth day of illness, and the titre of the reaction rises progressively from the time of the first appearance of agglutinins and reaches a maximum about the end of the third week. The reaction is usually positive in enteric carriers and in those who have been immunised with T.A.B. vaccine. A rising titre denotes infection and is therefore highly significant in diagnosis. It has been claimed that in inoculated persons the agglutinins are mainly of the H type, whereas both H and O agglutinins develop in the serum of infected persons; but it has been shown that both H and O agglutinins develop as a result of immunisation with T.A.B. vaccine and in such cases the diagnosis of typhoid fever cannot be made on the presence of O agglutinins only. Much importance attaches to the demonstration of the Vi agglutinin, as this depends upon the presence of the living organism in the body and is thus indicative of infection. Normal serum may agglutinate typhoid and paratyphoid bacilli; hence no significance attaches to positive Widal reactions within normal limits. These may vary in different countries and communities, but for Great Britain the normal agglutination figures are taken as:—*B. typhosus* H, 1 in 30; O, 1 in 50; *B. paratyphosus* A: H, 1 in 10; O, 1 in 10; *B. paratyphosus* B: H, 1 in 30; O, 1 in 50.

Technique. The following method for standardising diagnostic agglutination tests is that of Dreyer, adopted by the Standards Laboratory, School of Pathology, Oxford University, from which standard bacterial suspensions can be obtained. A series of dilutions of the serum, bacterial suspension and saline are prepared in Dreyer's agglutination tubes. Each tube contains 0.6 ml. of bacterial suspension and the amount of serum and saline are adjusted to give a total volume of 1 ml. and the following final dilutions of serum:—1 in 25, 1 in 50, 1 in 100, 1 in 250, 1 in 500 and 1 in 1000; a control tube contains bacterial suspension and saline but no serum. The tubes are incubated in a water-bath at 50–53° with only one-third of the volume of liquid in the tubes being below water level. For H agglutination incubate for 2 hours and for O agglutination incubate for 18 to 24 hours, followed by 15 to 20 minutes at room temperature. The reaction is read with the naked eye by artificial light against a dark background. "Standard Agglutination Tubes" showing the amount of clumping known as "standard agglutination" are supplied.—*Spec. Rep. Ser. med. Res. Coun., Lond., No. 51, 1931.*

Bacterial Suspensions for H and O Agglutination. The O antigen is destroyed by formalin, hence formalin-treated cultures give an almost pure H reaction. 0.1% formaldehyde is added to a 24-hour culture in veal extract broth, or an agar culture is suspended in saline containing 0.1% formaldehyde. The H antigen is destroyed by alcohol; a smooth colony is subcultured on agar containing phenol 1 in 800. The growth is scraped off and suspended in a minimum amount of normal saline solution, emulsifying very carefully. About 20 times the volume of absolute alcohol is added and the mixture heated to 40°–50° for 1 hour. After centrifuging, the deposit is suspended in normal saline with chloroform as

preservative. If an old stock suspension is used in the Widal test it should be centrifuged and resuspended in saline.

The popularity of the Widal Test dies hard; it is necessary to state again and again that the Widal reaction is not the diagnostic method of choice. Besides its uselessness for early diagnosis, its pitfalls have been accentuated by the increasing number of the population who have received prophylactic T.A.B. inoculations. In the first week of infection blood culture is most likely to give the diagnosis.—*Lancet*, i/1942, 685.

Paratyphoid Fever, in the true sense, is an infection with paratyphoid A and B bacilli. The disease is similar to typhoid, though generally running a milder course. Intestinal ulcers are identical with those of typhoid. Cases of mixed infection are not rare.

Paratyphoid A and paratyphoid B bacilli are morphologically like the typhoid bacillus and are actively motile but ferment glucose with production of acid and gas.

B. paratyphosus A produces less gas in glucose media than B; with A, milk remains acid for a fortnight and then becomes alkaline; and though A changes neutral red to yellow, the red colour tends to return after 3 weeks or so, while with B the yellow colour is permanent. That is to say, in its reactions A is more closely allied to the typhoid bacillus than B.

B. paratyphosus B, however, ferments xylose and blackens a lead acetate medium, while A does not, and B is regarded as more closely allied to the Gaertner group than A.

Types of *B. paratyphosus* B. Strains of this organism have been classified into types according to the rate of acid production in three fermentation tests—fermentation of rhamnose and of inositol and the so-called Bitter Test.

Diagnosis. The value of faecal cultures in the diagnosis of paratyphoid fever was demonstrated in a group of 400 cases, 98% of which were confirmed in this way. This method was applicable at all stages of the infection. In 92% of the cases, cultures were obtained at the first attempt, in 7% at the second, and in 1% at the third. The rate of clearing of faecal infection was found to be particularly slow in the case of women over 20 years of age; of 26 women over 40 years of age, 9 continued to excrete paratyphoid bacilli in the faeces for more than sixteen weeks.

Account of an outbreak in Bristol during the summer of 1941, involving 244 people with two deaths, and traced to a carrier working in a bakery making artificial cream.—I. G. Davies *et al.*, i/1942, 129. Account of an epidemic in Liverpool, involving 466 cases, with six deaths.—H. D. Holt *et al.*, *Lancet*, i/1942, 133.

Rhamnose and Inositol Media. These contain 1% of peptone, 1% of Liebig's meat extract, 0.5% NaCl and 0.5% of the fermentable substance. Adjusted to pH 7.5 to 7.6. To each litre of medium add 12 ml. of indicator solution containing 1 g. of bromothymol blue to 500 ml. N/200 NaOH.

Bitter Test Medium. This contains 0.5 g. of di-sodium hydrogen phosphate, 1 g. of sodium citrate, 5 g. of sodium chloride, 0.05 g. of peptone, 5 g. of rhamnose in 1 litre of distilled water. pH about 7. Indicator, methyl red in 96% alcohol.

All these media are inoculated with a large loopful of 18 hours' broth culture. The result of the rhamnose test is read after 12 hours' incubation (yellow = positive). After 15 hours' incubation 2 to 3 drops of the methyl red solution are added to the Bitter medium (orange red to purple = positive). The inositol test is read after 18 to 24 hours (yellow = positive).—Mackie and McCartney.

Tetrathionate Broth. 10 ml. of 60% solution of sodium thiosulphate and 5 ml. of 30% solution of iodine in 25% potassium iodide are added to 90 ml. of broth containing 2.5% chalk. The thiosulphate must be added before the iodine, and the chalk should be sterilised in the broth.

Experimentally these concentrations were found highly suitable for *B. paratyphosus* B, but for *B. typhosus* it is perhaps better to use a 50% solution of

thiosulphate and 25% solution of iodine. When stored at room temperature in the dark, the selectivity of the medium appears to remain unaltered for a week and declines very little up to two weeks; thereafter it is less satisfactory. If it is stored in the refrigerator, a precipitate may form. The optimum quantities of thiosulphate and iodine vary somewhat with the basal medium used, and the selectivity seems to be better with slightly alkaline (pH 7·6) than with neutral or acid media. The amounts of thiosulphate mentioned are in large excess of the amount required to combine with the iodine and should not be exceeded; they may perhaps with advantage be reduced.—H. D. Holt *et al.*, *Lancet*, i/1942, 133.

Paratyphoid C. Paratyphoid C differs from the other paratyphoid fevers in certain important respects. The causative organism is closely related to *B. suispestifer* and at one time the two organisms were considered identical. Outbreaks of paratyphoid C develop most readily in a community already affected by some other disease. The infection is essentially a septicæmia without involvement either of the intestine or of the mesenteric glands. The causative organism can be isolated from the blood without difficulty and at any stage of the disease. The fever may last from a few days to six weeks and there is a peculiar liability to localised septic complications in the form of cold abscesses. (*See also Bacterial Food Poisoning, this vol., p. 876.*)

Enteric Carriers

The carrier is the most important factor in the continued existence of typhoid and paratyphoid fevers as endemic diseases. Chronic carriers are people who, as a result of infection with typhoid or paratyphoid organisms (with or without accompanying illness), continue to excrete the bacilli in their faeces or urine, or both. Temporary carriers are those who excrete the organisms for no longer than a year from the date of infection. Excretion in the faeces is often intermittent; excretion in the urine is almost always continuous.

In the search for a carrier among a small number of suspected contacts, cultures of urine and faeces should be made on at least three occasions separated by a week. When the number of suspects is large, a preliminary selection by agglutinating tests is necessary. These tests may be against the H, the O and the Vi antigens. H agglutination tests are useless in people who have had T.A.B. vaccination or an authenticated attack of the disease, but may furnish useful indications in those who have not. O agglutination can arouse suspicions of the carrier state, even in vaccinated persons, provided vaccination is not of more recent date than about two months. A positive Vi agglutination test strongly suggests that the person is a chronic carrier. It may, of course, be positive during an attack of the disease, but only remains so for a short time after recovery. On the other hand, vaccination with vaccine hitherto in use does not produce Vi agglutination. A "Vi-positive" therefore demands a long cultural examination with all the available adjuvants (including at least two examinations of duodenal juice containing bile) before freedom from infection is reported. Negative reaction to the Vi test, on the other hand, can be dismissed with almost complete safety in a preliminary survey since not more than 5% of true chronic carriers will give a negative Vi reaction.—W. M. Scott, *Lancet*, i/1941, 389.

Bacteriophage Typing of *B. typhosus*. A bacteriophage specific for the Vi form of *B. typhosus* was described by Craigie and Brandon (*J. Path. Bact.*, 1936, 43, 233); Craigie and Yen (*Canad. publ. Hlth J.*, 1938, 448 and 484) elicited four serological types of Vi-specific phage, one of which (Phage 2) seemed to attack different strains of the typhoid bacillus with varying intensity. Phage 2 when propagated on a certain strain develops a high lytic activity for that strain but not for other unrelated strains. Extension of this observation resulted in the differentiation of a large collection of *B. typhosus* into a limited number of groups tentatively labelled Types A to J. By a fairly simple technique 98·6% of 592 typable cultures have been assigned to one or other of the six main types. Of over 700 cultures only about a tenth of them could not be typed because they were "imperfect Vi forms" whilst a smaller number did not contain any Vi antigen. *In vitro* tests for stability of these types failed to show any lack of type stability. The significance of these observations is that the isolation of the same

type of *B. typhosus* from a carrier, and from a series of patients whom the carrier is suspected of having infected, would add strong bacteriological support to the epidemiological evidence.—*Lancet*, ii/1938, 1476.

We have in the Vi agglutination test a promising method of detecting permanent carriers and in Vi bacteriophage an extremely delicate and accurate means of studying certain aspects of the epidemiology of the disease.—J. S. K. Boyd, *Brit. med. J.*, ii/1939, 902.

Treatment for Carriers. Oral treatment with soluble iodophthalein of 65 bile carriers sterilised the bile in 32.3% and freed the stool from typhoid bacilli in 7.6% of the carriers. 4 g. of soluble iodophthalein was given orally in orange juice three times a week to a total of 40 doses, and the patients kept on a low fat diet. This treatment should be given consideration before cholecystectomy is resorted to, particularly in bile carriers past 50 and in the presence of a well-functioning gall-bladder.—W. Saphir, *et al.*, *J. Amer. med. Ass.*, 1/1942, 964.

Typhus Fever. See *Rickettsia* Diseases.

Undulant Fever. *Syn.* MALTA FEVER, MELITENSIS FEVER, ABORTUS FEVER (see also p. 870). Originally the term "undulant fever" referred to the disease resulting from infection with *Brucella melitensis*, but it is now common to apply it to infections caused by *Brucella abortus* and *Brucella suis* which resemble melitensis fever very closely. In Great Britain undulant fever is always caused by *Br. abortus* (bovine). In 1886, Bruce found *Micrococcus melitensis* in the spleen of fatal cases of Malta fever, and by inoculating monkeys proved it to be the cause. *Br. abortus* was differentiated by Bang in 1897, and *Brucella suis* by Traum in 1914. Goats are particularly liable to harbour the organism and to excrete it in their milk. The disease is usually contracted by humans by consumption of infected milk, butter or cheese; it may also occur from direct contact with infected animals or their excreta. The organism can enter the skin through cuts or abrasions and may survive for several weeks in dust, soil or water.

The incubation period of from six to fifteen days is followed by headaches, insomnia, malaise and anorexia. The temperature, of a remittent type, gradually rises to 105°F. or higher, and this rise is usually accompanied by profuse perspiration with pains and sometimes swellings in the joints. Constipation is the rule and there is early splenomegaly. The tongue is flabby and coated with a silvery fur, the tip and edges remaining clean. After rising for three or four days by a step-like ascent the temperature falls in a similar manner and reaches normal by about the tenth day, after which the symptoms abate for a few days, only to be followed by a relapse. These relapses continue to follow each other at short intervals (thus producing the typical undulant fever chart), the disease running an average course of sixty to seventy days, though it may extend to a year or even longer, and no patient should be regarded as convalescent until the temperature and pulse have been normal for at least a fortnight and all other symptoms have disappeared. The mortality rate ranges from 2 to 10%. In malignant cases, which are characterised by sudden onset, with diarrhoea and vomiting, and with continued high fever, death generally results within three weeks.

Morphology. *Brucella melitensis* (*Micrococcus melitensis*) is a short and slender bacillus; the axis is straight; the ends are rounded; the sides may be parallel or convex outwards. In length the bacilli vary from about 0.6 to 1.5 μ and in breadth from 0.5 to 0.7 μ . The short forms may appear as oval cocci or as diplococci. As a rule they are arranged in pairs end-to-end, or in small groups; sometimes short chains of four to six members may be seen. Owing to the frequent coccoid appearance their bacillary nature may be in doubt, but in size they are smaller than any of the Gram-negative cocci. It is Gram-negative, non-motile and does not ferment any sugar. It will grow slowly in ordinary media but more rapidly in the presence of glucose, blood-serum or liver extract. *Br. melitensis* is closely related to *Br. abortus* and *Br. suis*, from which it may be differentiated by absorption tests. Whereas *Br. melitensis* and *Br. suis* grow well in ordinary atmospheric air, *Br. abortus* (*bovini*) will only grow in the presence of increased CO₂. The optimum atmospheric conditions are air containing 20% O₂ and 5-10% CO₂. The three organisms differ in their susceptibility to the inhibitory action of dyes, e.g., thionin, basic fuchsin and methyl violet.

Incidence. Undulant fever should not be regarded as a last resort in diagnosis but as a definite possibility in all cases of irregular or prolonged fever. Details of cases asked for, for the records of the Ministry of Health and the Agricultural Research Council.—Sir W. Dalrymple-Champneys, *Lancet*, i/1934, 95.

Of 1331 sera examined at Manchester for the *Brucella* and enteric groups between January 1929 and November 1932, 28 agglutinated *Br. abortus* only. Evidence is accumulating to show that latent and sub-clinical infection is not uncommon and the low incidence must depend on the relative insusceptibility of the human population.—E. Wade, *Lancet*, i/1933, 1342.

In the years 1928-34 inclusive there were 9317 cases of undulant fever in the United States of America; there were 318 cases in England in the years 1926-35, in France, 1932-34, 1109 cases; Germany, 1930-35, 3170 cases; Italy, 1924-35, 18,086 cases; Holland, 1930-35, 148 cases. Since the vast majority of cases are unrecognised, it is impossible to estimate the extent of brucellosis in man and in animals. No correlation exists between the number of cases of infected cattle herds with Bang's bacillus and the corresponding number of cases of undulant fever in man.—L. Greenfield and D. C. A. Butts, *Amer. J. med. Sci.*, 1937, 194, 678.

Diagnosis. Diagnosis is best established in the early stages of the disease by blood culture, and in no disease is bacteriological investigation more important. Agglutination tests are relied upon for diagnosis later in the disease. Burnet's intradermal test is also of value but is less reliable than the agglutination reaction. The disease should be suspected in all cases of fever of unidentified origin of more than seven days' duration with a reduced or normal white-cell count.

The laboratory methods which are regarded as most useful in the diagnosis of undulant fever are:—

(1) *The Brucellergen Intradermal Test.* This is performed by injecting 0.1 ml. of a suspensoid of nucleoprotein (1:2000) isolated from *Brucella* cells, intradermally on the forearm, and is read after 48 hours. (2) *The Opsonic Test.* This is performed by incubating a mixture of a live 48-hour culture of *Br. abortus* and the patient's citrated blood in a water-bath at 37° for 30 minutes. The mixture consists of 0.1 ml. of a saline suspension of organisms having a turbidity of 6 mm., as measured by the Gate's apparatus, and 0.1 ml. of the patient's citrated blood having a dilution of 0.8% citrate. A smear of the mixture is then made on a glass slide, rapidly dried with an electric fan and treated with 0.5 ml. of Hasting's stain (a modified Romanovsky stain) for 30 seconds, after which 1 ml. of distilled water having a pH of 6.4 is added for 10 minutes. Twenty-five polymorphonuclear leucocytes are examined and their opsonic power classified according to the number of *brucella* organisms counted within each cell, as follows: negative, no phagocytised bacteria; slight, from 1 to 20; moderate, from 21 to 40; and marked, over 40. (3) *The Rapid Agglutination Test*, and (4) *Culture of Brucella.* The brucellergen test is the most sensitive, and if this test is negative, brucellosis will usually be ruled out; if the test is positive, the opsonic test should then be performed to determine whether infection or immunity is present. The agglutination test is diagnostic in only a small percentage of cases and gives no information as to the immune status of the subject.—S. E. Gould and I. F. Huddeson, *J. Amer. med. Ass.*, ii/1937, 1971.

Agglutination Test. In the blood of normal persons agglutinins may be present for *Br. melitensis*, sometimes up to a titre of 1:50, occasionally to 1:100. In patients suffering from Malta fever, specific agglutinins appear about the tenth or twelfth day after the commencement of the fever; the titre rises till it attains a point varying between about 1:100 and 1:3000. Provided that the precautions are taken of using known agglutinable strains of both serological types, that no reaction is considered possible in a serum dilution of less than 1:50, and that in all doubtful cases the reaction is repeated in five days' time to ascertain if the titre is rising, there is little chance of error.—Topley and Wilson.

A titre of 1:80 or less in the absence of clinical symptoms indicates either a latent *Brucella* infection or a past infection. A titre 1:80 or more in the absence of clinical symptoms or of a recent pyrexial attack, is suggestive of frequent infections usually in persons drinking large quantities of infected raw milk or exposed to contact with infected animals. A titre of 1:80 or over in the presence of pyrexia and other symptoms, in persons whose habits or occupation do not involve special risk, is very suggestive of active infection. A titre of 1:20–1:80 in the presence of clinical undulant fever is practically diagnostic. The complete absence of agglutinins from a patient's serum does not exclude a diagnosis of *Brucella* infection.—Topley and Wilson.

Intradermal Reaction. Burnet's intradermal "Melitene" Test consists in injection of 0.5 ml. of a killed broth-culture containing 500,000 organisms. If positive, a red oedematous area occurs at the site of injection persisting for several days. Results should be read 96 hours after injection.

Cultivation. In most cases it is possible to culture *Br. melitensis* from the blood, which should be taken at the beginning of a febrile wave or at the height of the daily rise of temperature. The blood should be inoculated with glucose broth, which should not be discarded as negative within a period of at least one month. Blood culture is best made as early as possible in an attack. The organism is usually present in the urine at some time, especially during desferescence, but repeated cultures may be required for its detection. The best culture medium is 1% glucose broth and incubation prolonged in an atmosphere of 10% carbon dioxide.

Treatment. There is no specific treatment. Careful regulation of the diet so as not to overtax the digestion, supplemented by vitamin concentrates. Febrifuge drugs are contra-indicated. Autogenous vaccine are sometimes effective in doses of 25 to 50 million organisms, intramuscularly, gradually increased. Convalescent serum is often useful. Protein shock therapy by intravenous injections of T.A.B. vaccine sometimes shortens an attack.

Brucella melitensis vaccines containing 2000 million *Br. melitensis* organisms per ml. have been used in the treatment of undulant fever. The vaccine is given subcutaneously at intervals of from 3 to 10 days in doses of 0.25 ml., 0.5 ml., and 1 ml.

Ten cases treated by typhoid vaccine intravenously, with satisfactory results in every case. No recurrences and the follow-up agglutination tests for *Br. abortus* were negative in 9 of the 10 cases treated.—C. K. Ervin, H. F. Hunt and J. S. Miles, *Amer. J. med. Sci.* 1936, 192, 234.

Successful results following the use of mixed typhoid vaccine intravenously. The benefit probably comes chiefly from the fever induced, but the stimulation of antibodies may also be important.—C. E. Ervin and H. F. Hunt, *J. Amer. med. Ass.*, ii/1937, 1966.

Brucella infection should be suspected in cases of atypical meningitis. *Brucella* meningitis can be treated by human immune serum obtained from individuals who have been actively immunised to *Brucella*, or possibly from convalescent cases in the community who have high agglutinating titres in their blood serums for the specific organisms isolated from the spinal fluid. Patients who have no agglutinins for *brucellæ* in their blood after recovery should be immunised with their own organism to prevent a recurrence of the infection. Two cases of *brucella* meningitis are described that recovered rapidly and completely after intrathecal injection of fresh human immune serum.—M. A. Poston and D. T. Smith, *New Engl. J. Med.*, ii/1936, 369.

Weil's Disease. *Syn.* SPIROCHÆTAL JAUNDICE, INFECTIOUS JAUNDICE, LEPTOSPIROSIS ICTEROHÆMORRHAGICA. An acute febrile disease with world-wide distribution caused by *Leptospira icterohæmorrhagiae*. The incubation period varies from six to

twelve days. The onset is acute, with rigors, vomiting, headache, pain in the back and limbs, and irregular fever (103° to 105°F.) falling by lysis on the tenth to eleventh day. Conjunctival injection (pink eye) is present in a high percentage of cases and is of considerable diagnostic importance. Jaundice appears about the fourth or fifth day and deepens until the ninth or tenth day, when it fades away; the colour of the skin is lemon-yellow, darkening later to orange. With the onset of jaundice the urine becomes dark brown and contains albumin, renal casts, pus cells, bile salts and bile pigments; the stools contain excess of fat and are often pale brown or even white in colour. Constipation is the rule. There may be some enlargement of the liver and spleen and the gall-bladder is distended. A neutrophil leucocytosis is characteristic. The tongue becomes very dry, brown and fissured, and bleeding gums, epistaxis, hæmoptysis, hæmatemesis and melæna have been noted. The symptoms begin to subside about the third week though there is not infrequently a relapse at this time lasting from one or three weeks. The mortality rate is about 15%; the disease appears to be more severe and more often fatal in hot countries. Milder forms of the disease occur which may or may not be associated with jaundice; in all, however, injected conjunctivæ and a trace of albumin in the urine appear to be constant signs.

Morphology. *Leptospira icterohæmorrhagica*, the causal organism, is 6 to 9 μ in length, 0.25 μ in thickness, and contains a number of perfectly regular, closely-wound spirals about 0.5 μ long; the spirals become closer near the extremities. A most characteristic feature is the sharp, tapering, hooked ends, giving the organism a resemblance to the letter C or S.

Transmission. Infection of human beings takes place by contamination of the abraded skin or the conjunctiva with water contaminated with rat's urine. Canal workers, bargemen, sewer workers and coal miners are liable, and infection may result from bathing in infected water. Spirochætal jaundice is now officially classed as an occupational disease and comes under the provisions of the Workmen's Compensation Act.

A widespread and hitherto unrecognised focus of Weil's disease affecting the sewer labourers of London has been demonstrated, and its existence for at least 12½ years conclusively proved, many cases having been diagnosed as catarrhal jaundice.—N. H. Fairley, *Brit. med. J.*, ii/1934, 13.

Nineteen cases reported in Aberdeen, in fifteen of which clinical diagnosis was confirmed by bacteriological or serological tests. Thirteen of the patients were employed in the handling and cleaning of fish. The premises where fish is cleaned and prepared for distribution are often unsatisfactory, unhygienic, and infested with rats, and samples of water taken from floor-washings and tubs caused typical icterohæmorrhagial infection in guinea-pigs, the organism being easily demonstrable.—L. S. Davidson and co-workers, *Brit. med. J.*, ii/1934, 1137.

Twenty-four out of 210 fish workers in Aberdeen gave positive serum reactions, whereas 406 samples of blood from non-fish workers gave no positive serum reactions. Twenty-four per cent. of the rats trapped in the harbour district of Aberdeen were infected with *Leptospira icterohæmorrhagica*. Samples of water taken from the floor washings of establishments where Weil's disease had occurred produced typical icterohæmorrhagial lesions when injected into guinea-pigs, the organism being found in the liver and kidneys. The death rate in Aberdeen was only 6-7%—considerably lower than generally found in Europe, probably because the majority of the fish workers in Aberdeen were healthy young girls 15-20 years old.—L. S. P. Davidson, *Glasg. med. J.*, 1938, 129, 113.

Cases of Weil's disease in tripe-workers at Glasgow.—R. D. Stewart, *Lancet*, i/1938, 603.

Diagnosis. The leptospira are present in the blood during the first few days of the febrile period but are usually few in number and it is generally

necessary to inoculate a guinea-pig (3 to 5 ml. intraperitoneally) to demonstrate their presence; after the death of the animal they will be found in large numbers in the liver. After the first week of the disease the leptospira should be searched for in a centrifuged specimen of urine; they are best seen by dark-field illumination.

Agglutination Test. This is carried out by allowing varying concentrations of the patient's serum to interact for 2 hours at 32° with an equal volume of a young culture of *L. icterohæmorrhagiae*. Drops from these dilutions are then examined by dark-ground illumination. If formalised cultures are used, lysis of the leptospira does not occur. The technique of the reaction is described by W. Schüffner, *Trans. R. Soc. trop. Med. Hyg.*, 1934, 7.

Positive agglutination reaction may be expected on the sixth day of the disease and reaches its maximum on the twentieth.

A macroscopic agglutination test for diagnosis of Weil's disease.—J. Smith and W. J. Tulloch, *Lancet*, ii/1937, 846.

Adhesion Test. About 20 c.mm. of each of (a) patient's serum, (b) young broth culture of *L. icterohæmorrhagiae*, (c) saline suspension of young culture of *B. coli* or similar organism and (d) a five-fold dilution of fresh guinea-pig's serum in saline are mixed in a small incubation tube and incubated at 37° for half an hour. A control tube containing normal serum in place of the patient's serum is similarly treated. A drop of the fluid is examined under a cover slip with dark-field illumination. In a positive reaction the bacteria will be seen to be firmly adherent to the leptospira although not all the leptospira will be affected in this way unless the serum is of very high titre. In a negative reaction the leptospira are seen to be swimming freely unimpeded by the bacteria. At least twenty leptospira should be observed before a negative result is recorded.—H. D. Brown, *Lancet*, i/1935, 411. Culture medium used is composed of distilled water 3 ml., Lemco broth 0.5 ml., inactivated rabbit's serum 0.25 ml. Culture incubated at 32° for 6 days. Better to use a strain that has lost its pathogenicity.—*ibid.*

Treatment. The protein content of the diet should be minimal and fluids and dextrose given in large quantities. Intravenous dextrose (5%) and citrates are of special value. Specific therapy, both in the form of convalescent serum and antileptospiral serum is available.

Whooping Cough. The causative organism of the disease is generally considered to be the bacillus of Bordet and Gengou, *Hæmophilus pertussis*, which occurs in the form of minute oval rods, and closely resembles *B. influenza* (Pfeiffer). It is Gram-negative and non-motile.

Variation. Leslie and Gardner (*J. Hyg., Camb.*, 1931, 423) studied the antigenic properties of thirty-two strains, and divided them into four groups which they designated Phases I, II, III and IV. The freshly isolated (smooth) strains were of Phase I and were toxic for animals, while the old (rough) strains fell into Phases III and IV and were non-toxic. Furthermore, they were able to produce all four phases from a single strain by cultural methods showing that the types which had been previously described were merely variants. In culture the bacilli tend to change to Phases III and IV. This change can be delayed by incorporating sufficient fresh blood in the culture medium. An efficient prophylactic vaccine can be made only from bacilli in Phase I.

Complement-Fixation Test. As the disease progresses the patient's blood acquires complement-fixation substances, agglutinins and precipitins against *H. pertussis*. Because they do not reach their full amount until the fourth to sixth week of the disease, tests for their presence can hardly be utilised as a diagnostic measure, though such tests have their use in assessing the success of prophylactic treatment with vaccines or in determining whether an individual has a natural immunity to whooping cough.

The antigen used was a 1:5 dilution of a whooping cough vaccine containing 4000 million *H. pertussis* per ml. 0.25 ml. of this diluted antigen was added to each of four test-tubes and to each of these, and to a control tube containing 0.25 ml. of normal saline solution, the patient's serum was added in amounts which varied from 0.5 ml. in the first series of cases up to 0.2 ml. in the later batches of sera, this latter quantity of serum having been considered to be the optimum amount for the test. The complement dose of fresh guinea-pig serum was estimated for 0.5 ml. of sensitised sheep's corpuscles (3%), and 2, 3, 5 and 7 doses of complement were added to tubes 1, 2, 3 and 4, and 2 doses to the control tube. In the later complement-fixation tests, the fourth tube containing 7 doses of complement was omitted. The tubes and their contents were incubated for 1½ hours at 37° after which 0.25 ml. of sensitised sheep's corpuscles (3%) was added and incubation continued for a further hour at 37°. In each batch of tests, serum and antigen controls were put up and a positive human or rabbit serum was included in the tests.—J. P. T. Paton, *Lancet*, i/1937, 132.

Intradermal Test. In carrying out the test 0.1 ml. of vaccine (e.g., Sauer's) was injected intradermally on the flexor aspect of the forearm, the proper antigen control being used for each different type of vaccine. The readings were taken when the reactions were at their height or usually 18 hours after the injection. A skin reaction was considered positive when the area of redness was 1 cm. or more in its longest diameter the control test being negative. An examination of the diagnostic value of the intradermal test using a number of different vaccines showed that the test failed to give specific results. A high percentage of patients with whooping cough gave positive reactions, but so did a group of control patients with no history of whooping cough. There was no correlation between the intradermal and the complement-fixation reaction.—J. P. T. Paton, *Lancet*, i/1937, 132.

Results of the intradermal test on 1300 cases of whooping cough failed to support the claim that the intradermal response to Sauer's vaccine (10,000 million organisms per ml.) is of value in demonstrating immunity to whooping cough or in the early diagnosis of the disease. The intradermal response to pertussis endotoxin on the other hand produces a reaction resembling that of the Schick and Dick tests and is of some value in assessing the state of immunity and as a diagnostic reaction in early, atypical or late whooping cough.—A. K. Thompson, *J. Hyg., Camb.*, 1938, 28, 104.

An endotoxin derived from *H. pertussis* Phase I can be used as a skin test specific to pertussis, and bears the same relationship to pertussis as does the Schick Test to diphtheria. The endotoxin is stable over a period of months and produces positive skin reaction by virtue of its primary toxicity. The best dose is 0.1 ml. of a 1:600 dilution which is equivalent to 1/20th M.L.D. for mice. The reaction should be watched between the sixth and twenty-fourth hour, as the erythema which develops, measuring from 1 to 3 cm. in diameter, may come on early and fade rapidly or be delayed and not show up for 24 hours.—L. P. Strean, *Canad. med. Ass. J.*, 1940, 42, 525.

Cultivation. For primary cultures blood or ascites agar or, preferably, a potato-glycerin-blood-agar medium (*vide infra*) is used; the organism grows somewhat scantily on blood agar. Cultures can be made from sputum in the ordinary way or by the cough-plate method. The colonies develop rather slowly, at first resembling those of the influenza bacillus, and after 48 hours becoming slightly larger and thicker. After cultivation on artificial media for a time it can be made to grow on plain media.

Bordet-Gengou Medium. The following medium permits of isolation from sputum. Potato 500 g., 4% glycerin solution 1000 ml.; autoclave and pour off excess fluid. Emulsify potato in normal saline 1500 ml. and add agar 3 to 4%. For use, mix with equal quantity of defibrinated blood.

Cough-Plate Method of Diagnosis. The bacteriological diagnosis of whooping cough by the cough-plate method can be adopted for routine use. The basis of the method is a suitable medium, and the best results are obtained with the Danish modification of the Bordet-Gengou medium, as used in the Danish State Serum Institute. This is prepared as follows:—

Clean and pare potatoes and cut into thin slices. Add 250 g. of the slices to 500 ml. of tap water and 9 g. of NaCl. Boil till the slices fall to pieces, replace the water lost in boiling, and filter through linen. Adjust the reaction of the extract to pH 7.0. Add 500 ml. of the extract and 20 ml. of glycerin to 60 g. of agar (Bacto) dissolved in 1500 ml. of tap water. Distribute in flasks,

autoclave, and store till required. Cool the melted agar to about 48° and add an equal quantity of fresh defibrinated or oxalated horse blood warmed to about 40°. Mix well and place in a water-bath at 48° for 3 to 5 minutes. Pour into Petri dishes, avoiding bubbles. Incubate one plate to test for sterility; inoculate another with a stock strain of *H. pertussis* as a control of growth production. It is essential to have the medium fresh; a useful guide is the colour of the medium which should be a bright cherry red; a dark medium should be discarded.

In exposure of the cough-plate the natural spasm is essential. Usually plates should be exposed for 10 to 15 seconds and about 6 inches away so that no mucus reaches the plate, but in older subjects with much catarrh this may cause too heavy an inoculation, and in infants the plate may be held as close as three inches from the mouth. A useful prescription of the actual number of coughs is as follows: over five years, 6 to 8 coughs; three to five years, 8 to 12 coughs; one to three years, 12 to 16 coughs; and under one year, 20 to 30 coughs. In the early stages it may be necessary to stimulate coughing by means of a cold drink and direct stimulation of the epiglottis, and in the early catarrhal phase in infants it may be necessary to take pharyngeal swabs as an alternative. Routine examination comprises two plates usually taken on the same day, and if these are negative further plates are taken.—E. A. Straker and J. S. Westwater, *Lancet*, ii/1937, 565.

Isolation of the Bordet-Gengou bacillus by the cough plate is the only reliable method of securing a prompt and accurate diagnosis, as the organism is constantly found in whooping cough (and in no other disease) in the early stages of the disease and rapidly disappears during the fourth and fifth week—by the sixth week the great majority are bacilli-free. Experience of the method has shown that two negative plates taken on consecutive days provide sufficient warrant to release the patient from isolation, even although he is whooping violently.—William Gunn, *Practitioner*, ii/1936, 520.

The use of penicillin for the isolation of *H. pertussis* in cough-plates gives excellent results and saves much labour. Six to eight drops of penicillin are spread over one half the plate and the other half is left untouched; of 50 such cough-plates exposed, 47 gave positive cultures of *H. pertussis* on the penicillin side against 33 positive cultures on the untreated side. Penicillin is useful in another way—swabs taken from whooping cough cases only give 10% of positive results because the secondary growth is so great; but if the swabs are treated with penicillin the secondary growth is so much reduced that 75-80% of positive results are obtained.—I. H. Maclean, *Lancet*, ii/1940, 345.

Yaws. *Syn.* FRAMBOESIA. A contagious inoculable disease characterised by an indefinite incubation period followed by fever, by rheumatic pains, and by the appearance of papules which develop into a fungating, encrusted, granulomatous eruption. The disease is practically confined to the tropics and almost exclusively to coloured races.

The causal organism of yaws is *Treponema pertenue* (*Spirochaeta pertenue*) which morphologically is practically identical with *Treponema pallidum*. The primary lesion is of non-venereal origin and infection usually occurs in childhood, the organism entering the skin through abrasions or cuts, most commonly on the legs. Bejel (*q.v.*), the non-venereal syphilis of the Bedouin Arabs, has certain epidemiological and clinical similarities to yaws. The disease is not transmitted congenitally and infection is regarded as occurring only once in a lifetime; it is rarely fatal.

H. W. Kumm has brought forward strong evidence that the minute fly, *Hippelates pallipes*, is the active carrier of the causative organism, *Treponema pertenue*. He has collected these insects at the rate of 5000 flies per hour on one yaws ulcer alone. Flies will crawl under the scab and ingest large numbers of spirochaetes, which survive for seven hours in the anterior part of the gut and are probably transmitted by regurgitation.—Rep. Jamaica Yaws Commission for 1934, per *Med. Annu.*, 1937, 573.

Diagnosis. The Kahn, Wassermann and other serological tests used for the diagnosis of syphilis all give positive reactions with the serum of patients suffering from yaws. All the methods of staining commonly used for *T. pallidum* can be used for *T. pertenue*.

Treatment. The condition responds rapidly to neosarsphenamine intravenously or acetarsol per os.

Yellow Fever. A highly dangerous, acute febrile disease due to an ultramicroscopic filter-passing virus, transmitted by the mosquito, *Aedes aegypti* (except in the case of jungle yellow fever, *q.v.*). It occurs in endemic or epidemic forms in West and Central Africa and in South America. Yellow fever resembles Weil's disease in some respects, but the symptoms are more severe and hæmorrhages into the stomach and intestine cause black vomit and melæna. The average mortality rate is from 20–30%.

Morphology. The diameter of the virus is from 17–28 m μ . All strains of the virus show two major tissue affinities—viscerotropic and neurotropic; when both are present it is known as "pantropic" virus. By repeated passage of the virus through mouse brain the neurotropic properties can be greatly increased and the virus is no longer capable of producing viscerotropic lesions. Under certain laboratory conditions its viscerotropic properties can be restored. The virus can be grown on the chorio-allantoic membrane of the chick embryo. It can be concentrated by high-speed centrifugation. The virus so obtained is inactivated by distilled water and normal saline solution, hence suspensions should be made in monkey serum or in ascitic fluid. Both neurotropic and viscerotropic strains are inactivated by a temperature of 60°–65°, and by photodynamic action and dyes such as methylene blue or proflavine. They can withstand strong disinfectants such as mercuric chloride (1 in 7500) and phenol (1 in 150). The optimal temperature for the development of the virus in the body cavity of the mosquito is 26°, the limits being 18°–37°. The time taken for the virus to develop in the body of the mosquito varies with the temperature—at 37° 4 days suffice, at 21° 18 days; at 10°–15° the virus persists in the body of the mosquito without its bite being infective.

Epidemiology. For three centuries at least yellow fever has periodically ravaged the larger seaboard towns and cities of West Africa and tropical and sub-tropical America. Thence it has been transferred to Mexico, the United States, Canary Islands, Spain, Portugal and Italy and the small islands of Ascension and St. Helena in the South Atlantic. So far as is known it has never invaded Asia or Australia.

Two methods of diagnosis—viscerotomy and the mouse-protection test—demonstrated that many persons in S. America were dying of yellow fever in the absence of "visible" outbreaks of the disease, and that the distribution of the disease was much more extensive than had been thought. The mouse-protection test also demonstrated that the disease was occurring in the complete absence of the accepted vector *Aedes aegypti*. It has now been established that in South America to-day yellow fever is primarily a jungle infection of which *Aedes aegypti* is not the vector and that man is not an important reservoir of the virus. The present position in South America is that there are three types of yellow fever—the *aegypti*-transmitted urban and rural types, and the non-*aegypti*-transmitted jungle type. The first two are acquired indoors, are likely to infect non-immune persons of all ages and to spread along the routes of human travel. They are relatively readily controlled by anti-*aegypti* measures. The jungle type is only acquired by forest workers, does not spread to other members of the community in the absence of *Aedes aegypti* and spreads through the jungle irrespective of the routes of human travel. It can only be brought under control by the immunisation of exposed members of the population. Clinically, pathologically and immunologically the various forms of the disease are indistinguishable.

Somewhat the same position obtains in Africa, but it has not yet been established that jungle yellow fever exists there, although many facts strongly suggest that it does. The potential danger of the situation is illustrated by an outbreak in Kordofan province, where no case of the disease had ever been recognised clinically before October 1940, although mouse-protection tests had shown the

presence of the infection some years before. The epidemic—the greatest so far recorded in any part of Africa—lasted two months and involved an area of 6000 square miles. There were 15,000 recognised cases with 1600 deaths.—*Lancet*, i/1941, 453.

Diagnosis. In the average case fever associated with undue prostration and early and increasing albuminuria should at once arouse suspicion. The most distinctive sign is the increasing albuminuria. Albumin appears in the urine usually on the second or third day and reaches a maximum from the fifth to the seventh. In endemic regions pyrexia plus the development of heavy albuminuria is regarded as practically diagnostic of yellow fever. In the first three days of the fever the virus is readily transmitted by inoculation of 0.3 ml. of the patient's blood intracerebrally into white mice, in which the characteristic encephalitis is produced in 7 to 15 days. More frequently it will be necessary to take two specimens of blood at a fortnight's interval and establish a rise in the titre of protective antibody by the "mouse protection test" (*q.v.*) in order to establish the diagnosis. In fatal cases diagnosis can be made on the characteristic histological changes in the liver (viscerotomy).

Mouse Protection Test. The present method is to inject 0.03 ml. of sterile starch solution intracerebrally into mice to fix the virus. This is followed by the intraperitoneal injection of 0.2 ml. of a 10% emulsion of the brain of a mouse dying of yellow fever along with 0.4 ml. of the human serum to be tested. Six mice are similarly inoculated and various controls with known positive and negative sera. At least 5 of the 6 control mice receiving normal serum should die of yellow fever encephalitis in from 5 to 10 days.—Stitt.

Immunisation. An attack of yellow fever, even if so slight as to be clinically unrecognisable, confers a lasting immunity. The "mouse protection test" (*vide supra*) which has been used to study the distribution of the disease is based on the fact that the serum of an individual who has had, at some time, yellow fever, when injected into a mouse protects it effectively against an otherwise lethal dose of yellow fever virus.

Following the demonstration in 1930 that viscerotropic virus when rendered neurotropic by passage through mice loses its virulence but still confers immunity, two different series of vaccinations were begun. In both series neurotropic mouse virus was used at first, but in America it was made less potent by adding human (and later monkey) immune serum, whereas in Africa (Sellards and Laigret) it was given alone after attenuation by storage in glycerin. Subsequently Lloyd, Theiler and Ricci used viscerotropic monkey virus attenuated by culture for two years on embryonic tissue, still with the addition of immune serum. The latest development is by immunisation with attenuated pantropic virus, cultivated in serum Tyrode medium, containing mixed chicken embryo. At first the virus vaccine was mixed with human immune yellow fever serum, but since 1936 the virus alone has been injected into thousands of persons without any serious reaction.

In Africa during the past three years about 20,000 persons have been vaccinated against yellow fever, and not a single case of the disease has occurred in any of these subjects.—A. W. Sellards and J. Laigret, *per Trop. Dis. Bull.*, 1937, 688.

In Brazil where the most extensive immunisation scheme has been carried out, a pantropic virus "17 D" which has been cultivated on fowl embryo tissue since 1934 has been employed. During 1937 38,387 persons were vaccinated with this virus, and by the end of March 1938 the number had risen to 168,000. About 20% of the first 200 people inoculated at Rio de Janeiro exhibited symptoms attributable to the virus, but since then the reactions have been relatively mild. The virus used was grown in fowl embryos and mixed with human serum.—F. L. Soper, *Bull. Off. int. Hyg. publ.*, 1938, 30, 1197.

Between January 1st and July 4th, 1942, 28,585 cases of jaundice, with 62 deaths, occurred among Army personnel in the U.S. Army following the use of vaccination against yellow fever; between two and two and a-half million men were inoculated. The form of vaccine has now been modified.—*J. Amer. med. Ass.*, 1942, 119, 1110.

Treatment. There is no specific treatment. Convalescent serum has been tried but does not affect the course of the disease once symptoms have appeared. Careful nursing is essential and as much fluid as can be taken is given during the acute illness; it is vitally important to withhold all food during the early days of the illness. Citrates should be added to the drinks to combat acidosis. Intravenous injections of $\frac{1}{2}$ to 1 pint of 5% dextrose are of value.

TABLE OF INFECTIOUS DISEASES

| Disease | Incubation Period | Quarantine of Contacts | Isolation of Patient |
|--|---|---|--|
| Cerebrospinal Fever (Meningitis) | 3 to 4 days. | Nasopharyngeal swabs of doubtful value. Sleeping quarters spaced out and well ventilated. | Minimum of 14 days. |
| Chickenpox (Varicella) | 14 to 21 days. | Non-immunes (children only) kept under observation for 21 days. | Until all scabs have separated, leaving dry healed pocks. |
| Cholera | 1—3—6 days. | Suspects kept under observation for 5 days. | Until at least two consecutive bacteriological examinations of stools passed after a saline aperient, have proved negative. |
| Diphtheria | (i) <i>Faucial</i> , 1 to 3 days; (ii) <i>Nasal and Laryngeal</i> , indeterminate. | Immediate contacts kept under observation for a week or until Schick tests, swabbings and virulence tests are satisfactory. Under suitable circumstances a dose of antitoxin and a negative swabbing obviates quarantine. | Until mucous membranes are healthy and two consecutive negative swabs from nose and throat at intervals of not less than 3 days. Periodical virulence tests essential in cases of prolonged infectivity. |
| Enteric Fever (Typhoid and Paratyphoid) | (i) <i>Typhoid</i> , 12 to 14 days. (ii) <i>Paratyphoid</i> , 10 to 14 days. | Suspects kept under observation for 14 days, and preferably inoculated with T.A.B. | Until at least two bacteriological examinations of faeces and urine taken at intervals of a week have proved negative. |
| Epidemic Poliomyelitis | Very variable. From 5 to 21 days. | Immediate contacts kept under observation for 14 days. | 21 days from onset of pre-paralytic stage. |

| Disease |
|--|
| Epidemic Encephalitis |
| Erysipelas |
| Glandular Fever (Infective Mononucleosis) |
| Infantile Paralysis (<i>see</i> Epidemic Poliomyelitis) |
| Malaria |
| Measles |
| Mumps (Epidemic Parotitis) |
| Plague (Bubonic or Pneumonic) |

| Disease |
|---------------------------|
| Relapsing Fever |
| Rubella |
| Scarlet Fever |
| Smallpox (Variola) |
| Typhus (European) |
| Whooping-Cough |
| Yellow Fever |

The above table has

| Incubation Period | Quarantine of Contacts | Isolation of Patient |
|---|--|--|
| Variable 2—10—14 days. | Immediate contacts kept under observation for 14 days. | 7 days. |
| 1 to 3 days. | Nil. | Until local process has subsided and all wounds or abrasions have completely healed. |
| 5 to 12 days. | If imposed, 14 days. Infectivity very low. | 14 days minimum. |
| (i) Benign tertian, 14 days. (ii) Quartan, 21 days. (iii) Malignant tertian, 7 to 12 days. | Nil. | Nil. |
| (i) To catarrhal (Koplik's spots) stage, 10 to 11 days. (ii) To rash, 14 to 15 days. (iii) Artificially serum-attenuated measles more prolonged, e.g., 17 days to rash. | Non-immunes, 15 to 18 days. | 10 to 12 days, though infectivity has ceased by time rash has faded. |
| 14—18—21 days. | 21 days. | Minimum of 2 weeks, or 1 week after subsidence of last gland affected. |
| 2 to 8 days. | 10 days. | 1 month. |

| Incubation Period | Quarantine of Contacts | Isolation of Patient |
|---|--|--|
| 2 to 10 days. | Preliminary delousing and daily observation for 14 days. | Two weeks after last "relapse," if treated with organic arsenical preparations. |
| 14 to 19 days. | If imposed 21 days, but infectivity low compared with measles. | Until rash has disappeared, i.e., 4 to 5 days. |
| 1 to 3 days. Exceptionally 4 or 5 days. | 7 days, unless Dick tests and nose and throat swabbings satisfactory. Passive immunisation of all Dick positive immediate contacts with scarlet fever antitoxin controls outbreak for two weeks. | 4 weeks from onset if no discharges or other complications and mucous membranes healthy. Terminal swabbing for the hæmolytic streptococcus is unnecessary in these cases. Desquamation is not infective. |
| (i) Variola Major (Eastern strain) 12 days. (ii) Variola Minor (Western strain) 12 to 15 days. | If protected by vaccination keep under observation for 16 days; otherwise quarantine for 16 days. | Until all scabs have separated, leaving healed dry pocks. |
| 10 to 14 days | Preliminary delousing and then daily observation for 16 days. | Until at least a week after crisis, i.e. 21 to 28 days. |
| (i) To catarrhal stage, 7 to 14 days. (ii) To paroxysmal 21 to 28 days. | Non-immunes for 21 days from last exposure to infective patient (a child who has been whooping for a month is no longer infective). | 6 weeks from onset of catarrhal stage; 4 weeks from onset of paroxysmal stage. |
| 3 to 6 days. | 6 days. | 4 days screened in mosquito netting. |

CULTURE MEDIA FOR BACTERIOLOGICAL INVESTIGATION

Bile Salt Dextrose Broth [MacConkey's Medium (Double Strength) for *B. coli*]. Triturate 40 g. of peptone into a cream with water and add it to 1000 ml. of hot water containing 10 g. of sodium taurocholate. Boil the liquid for 30 minutes, cool, and add 10 g. of dextrose. Sterilise by steaming at 100° for 20 minutes, adjust to pH 7·4, filter through filter paper into a sterile flask, add sterile litmus solution to colour the medium to a deep purple. Place 10 ml. quantities into tubes containing small gas tubes and plug. Sterilise by steaming at 100° for 20 minutes.

(The medium is prepared with neutral red instead of litmus.)

Blood Agar [Citrate Blood Agar (Guy's)]. Kill a small rabbit with chloroform vapour, open up the thoracic cavity and pericardium, maintaining the strictest aseptic precautions throughout the operation. Insert a sterile plugged pipette into the heart. Apply suction to the plugged end and fill it with blood. Transfer the blood to a small sterile flask containing sterile glass beads and 5 ml. of a sterile 10% solution of sodium citrate in normal saline. Agitate thoroughly and set aside for 2 hours. Warm several tubes of sterile nutrient agar to 42° to melt and transfer aseptically 1 ml. of the blood to each, plug, and rotate the tubes so as to diffuse the blood evenly throughout the medium. Slope the tubes and allow to cool. Incubate at 37° and reject any contaminated ones.

Blood Serum (Loeffler). Prepare nutrient broth using a veal extract instead of beef. Dissolve 1% dextrose in the broth. Add 300 ml. of clear blood serum (prepared as for Blood Serum Medium) to every 100 ml. of broth. Tube and complete as for Blood Serum Medium.

Blood Serum Medium. Place freshly collected ox or sheep blood in a sterile cylinder and allow to stand for about 15 minutes until the blood has coagulated. Separate the clot from the sides of the vessel using a sterile glass rod, and place the cylinder in an ice chest for 24 hours.

Pipette off the serum in 5 ml. quantities into sterile tubes, and plug. Heat to 56° for 30 minutes on each of two successive days. On the third day heat the tubes in a sloping position in a serum inspissator to about 72° so as to form a transparent jelly-like coagulum (a higher temperature will give a turbid jelly). Incubate at 37° for 48 hours and reject those showing growth. Store the remainder in a cool place.

Brilliant Green Agar, N.F. Dissolve extract of beef 3 g., peptone 10 g., sodium chloride 5 g., and agar 15 g. in 1000 ml. of distilled water with the aid of heat, preferably in an autoclave. Add sufficient normal sodium hydroxide to make pH 7·4. If clarification is desired, filter the medium while hot through cotton enclosed in gauze. Place 100 ml. portions of the medium into suitable flasks and sterilise by heating in an autoclave for 20 minutes at 15 lbs. pressure. Dissolve dextrose 1 g. and lactose 10 g. in sufficient distilled water to make 50 ml. and sterilise by heating in an autoclave for 20 minutes at 15 lbs. pressure. Just prior to use, liquefy the medium by the aid of heat and aseptically add to each 100 ml. portion 5 ml. of the sterile dextrose-lactose solution, 1 ml. of sterile Andrade indicator and 0·2 ml. of a 1:1000 aqueous solution of brilliant green. Mix well and divide among six 100 mm. petri dishes. (*Andrade Indicator*: Dissolve acid fuchsin (70% dye content) 0·5 g. in distilled water 100 ml. and add normal sodium hydroxide in small portions to the solution until the colour changes from red to orange or yellow; about 16 ml. is usually required. Filter the solution and sterilise by heating in an autoclave for 20 minutes at 15 lbs. pressure. Culture medium containing Andrade indicator is pink when hot; at room temperature it is pink when acid, colourless at pH 7·2, and colourless or pale yellow when alkaline.)

Casein-Casein Yeast (C.C.Y.) Medium (Gladstone and Fildes). Dispenses with meat extract and peptone. Used in either solid (agar) or liquid form. A good substitute for meat infusion agar and broth. Most of the pathogenic bacteria will grow on it. For full details of method of preparation see G. P. Gladstone and P. Fildes, *Brit. J. exp. Path.*, 1940, 21, 161. The following are required:—1. *Hydrolysed Casein*: Casein 200 g., concentrated hydrochloric acid 170 ml., distilled water 110 ml.; stir quickly to make uniform

suspension before casein becomes solid; autoclave for $\frac{1}{2}$ hour at 120° . When cold neutralise with 40% caustic soda (about 180 ml.). Filter when cold through pulp on a Buchner funnel. Dilute to 1 litre; add 1% chloroform and shake vigorously at intervals till chloroform is emulsified. 2. *Tryptic Digest of Casein*. Casein 200 g., sodium carbonate 20 g., add tap water at 37° in two lots of 1 litre shaking after each addition. Add 100 ml. of a suspension of minced pancreas and 10 ml. chloroform. Shake. Adjust pH to 7.4-7.5. Incubate at 37° for 16 days, shaking daily. Keep pH adjusted throughout. Remove from incubator and stand 2 days. Filter off the white deposit of tyrosine crystals. To 2 litres of filtrate add 15 ml. conc. HCl in 150 ml. water; steam for 1 hour. Cool, add 40% NaOH (about 6 ml.) to make pH 7.4. Add 1% chloroform and shake to emulsify. 3. *Extract of Yeast*. Pressed brewer's yeast 250 g., boiling distilled water 1 litre. Crumble the yeast into the water (over naked flame), stir till frothing ceases. Filter through pulp in Buchner funnel. Add 1% chloroform. These solutions should be kept in stoppered winchester bottles in the dark. 4. *Sodium Glycerophosphate*. 5. *Glutamine*. 0.05% solution in distilled water; sterilise by filtration, keep in ice-chest; stock solution should be renewed at frequent intervals. 6. *Sodium lactate*. 50% solution of commerce.

To prepare the *solid medium* mix 15 g. agar in 850 ml. distilled water to make uniform suspension and steam in autoclave for 5 minutes; then increase pressure to 15 lbs. and turn off heat. Remove in half an hour. To the solution while boiling add a boiling mixture of hydrolysed casein 35 ml., tryptic digest of casein 15 ml., extract of yeast 100 ml., sodium glycerophosphate 10 g., sodium lactate 10 ml. Add about 3.6 ml. of 40% NaOH to make pH about 9.5 (to precipitate phosphates in commercial sodium glycerophosphate) and steam at not less than 90° - 95° . Allow the precipitate to settle, decant the liquid and filter rapidly through Buchner funnel; pulp and immediately add 2.05 ml. of HCl (32%, sp. gr. 1.142) per litre. Adjust pH to 7.6 (cresol red indicator), add 5 ml. of glutamine solution to each litre. Sterilise in autoclave.

The *liquid medium* is similar except that 850 ml. of water is used in place of the agar solution. Heating at an alkaline pH is not necessary, since in the absence of agar there is insufficient calcium to precipitate any phosphate present.

Egg Medium (Dorset). Wash twelve fresh eggs externally with water, then with solution of formaldehyde and finally in methylated spirit. Allow to dry. Break the eggs into a sterile graduated cylinder and measure the total volume of mixed whites and yolks. Add 1 volume of sterilised normal saline to 3 volumes of the mixed eggs. Transfer to a large, wide-mouthed, stoppered bottle, previously sterilised. Add sterile glass beads and shake thoroughly in a mechanical shaker for 30 minutes. Filter through muslin. Add, if required, a few drops of alcoholic solution of magenta. Tube in quantities of 10 ml. Heat the tubes in a sloping position in a serum inspissator at 75° to 80° for one hour. Incubate at 37° for 48 hours and reject any contaminated tubes. Cap the remainder with sterile rubber caps and store.

Glucose (Dextrose) Broth. Incorporate 20 g. of dextrose in 1000 ml. during the preparation of nutrient broth. Sterilisation should be carried out by steaming at 100° for 20 minutes on three successive days.

Glucose Agar Broth. Rub 20 g. of powdered agar into a smooth paste with 750 ml. of nutrient broth. Heat on a water-bath and pass steam through the medium until the agar is dissolved. Incorporate 20 g. of dextrose and warm gently until dissolved. Adjust to 1 litre with hot nutrient broth. Sterilise by steaming at 100° for 30 minutes on each of three successive days.

Glucose Trypsin Broth (Blood Cultures). Nutrient broth with 1% glucose. After filtering add Liquor Trypsini (A. & H.) in the proportion of 1 of liquor to 10 of broth.

Glycerin Blood Agar. Prepare blood serum as described under Blood Serum Medium and place in sterile tubes. Add 5% v/v of glycerin and proceed as for Blood Serum Medium.

Different percentages of glycerin are used for special purposes, but 5% is usually employed.

Glycerin Broth. Nutrient broth containing 6% v/v of glycerin.

Lactose-Bile-salt-Agar Medium (MacConkey's Neutral Red Bile Salt Agar). Rub 20 g. of peptone into a paste with 150 ml. of distilled water. Add 10 g. of lactose and 5 g. of sodium taurocholate dissolved in 500 ml. of

distilled water and bring the peptone into solution with gentle heat. Rub 20 g. of powdered agar into a smooth paste with some of the solution and incorporate the remainder. Heat on a water-bath and bubble steam through until the agar is dissolved; then adjust to 1 litre with boiling distilled water. Adjust the reaction of the medium to pH 7.0. Thoroughly whip the whites of two eggs and add to the medium. Steam at 100° for about 1 hour to coagulate the egg albumen and filter (using a hot-water funnel if necessary) into a sterile flask. Add 10 ml. of 0.5% w/v sterile solution of neutral red. Tube in quantities of 10 or 15 ml., plug and sterilise by steaming at 100° for 30 minutes on 3 successive days.

Nutrient Agar Medium. Rub 10 g. of peptone, 5 g. of sodium chloride, and 20 g. of powdered agar into a paste with 150 ml. of distilled water. Add 5 g. of Lab-Lemco dissolved in 500 ml. of distilled water and place in a flask on a water-bath. Bubble steam through until the agar is dissolved and adjust the volume to 1 litre with boiling distilled water. Determine the amount of sodium hydroxide required to adjust the medium to pH 7.4 and slowly add the necessary quantity of N/1 sodium hydroxide. Allow to cool to 60°. Thoroughly whip the whites of two eggs and add to the medium. Steam at 100° for about one hour to coagulate the egg albumen, and filter (using a hot-water funnel if necessary) into a sterile flask. Tube in quantities of 10 or 15 ml. and sterilise by steaming at 100° for 30 minutes on 3 consecutive days.

Nutrient Broth. 10 g. of peptone rubbed into a cream with water is added slowly to a boiling solution of 5 g. of meat extract (Lab-Lemco) and 5 g. of sodium chloride in 750 ml. of water. The liquid is autoclaved for 30 minutes at 115° and filtered, cooled and adjusted to 1 litre. An aliquot portion is titrated at 37° with N/10 sodium hydroxide until neutral to phenolphthalein and the calculated quantity of N/1 sodium hydroxide is then added very slowly to the remainder to render it neutral to phenolphthalein at 37°. The mixture is heated to boiling and filtered while hot. By the addition of N/1 hydrochloric acid the reaction is adjusted to pH 7.6 using phenol red as indicator. It is then placed in a plugged flask and autoclaved again.

The final reaction of the broth should be between pH 7.2 and pH 7.8.

Nutrient Broth for anaerobic test for sterility. Minced lean beef is boiled with water (containing a little sodium hydroxide to neutralise the meat acids) and the aqueous liquid decanted off. The residual fibre is repeatedly boiled with water until all fat and alkali are removed. The residue is drained free from liquid and placed to a height of 1 cm. in test tubes and nutrient broth for aerobic test is added, and the tubes are plugged and sterilised by autoclaving. After sterilisation the reaction of the medium must lie between pH 7.2 and pH 7.8.

Before using for anaerobic testing, the medium should be heated to 100° to free it from dissolved oxygen and then cooled.

Nutrient Broth for Rideal-Walker Test, see Standard Rideal-Walker Broth, p. 1015.

Nutrient Gelatin Medium. Rub 10 g. of peptone and 5 g. of sodium chloride into a paste with 150 ml. of distilled water. Add 5 g. of Lab-Lemco dissolved in 500 ml. of distilled water and to the mixture add 100 g. of sheet gelatin cut into small pieces. Heat on a water-bath and pass steam through the mixture until the gelatin has dissolved. Adjust the volume to 1 litre with hot distilled water. Ascertain the pH of the medium by titration of an aliquot portion with standard sodium hydroxide solution. Allow to cool to 60°. Thoroughly whip the whites of two eggs and add to the medium. Steam at 100° for about one hour to coagulate the egg albumen and filter into a sterile flask. Tube in quantities of 10 ml. and plug. Sterilise by steaming at 100° for 30 minutes on each of 3 successive days.

Peptone Water (Dunham). Rub 10 g. of peptone and 5 g. of sodium chloride into a smooth paste with 250 ml. of distilled water heated to 60° and adjust to 1 litre with cold distilled water. Steam at 100° for 30 minutes, filter through paper, tube in quantities of 10 ml. and plug. Sterilise by steaming at 100° for 20 minutes on 3 consecutive days. The pH need not be adjusted unless the medium is intended for the isolation of cholera vibrios when it should be adjusted to pH 8.4.

Potato Medium. Fairly large potatoes should be selected, well washed, and scrubbed with a stiff brush. Peel, and remove the "eyes". Using a large-sized cork borer (preferably silver-plated, because steel will cause discoloration), cut cylinders. Then slice each cylinder from end to end, forming

wedge-shaped pieces. Place each piece in a sterile test tube with a small plug of wet sterile cotton wool at the bottom. Plug and sterilise by steaming at 100° for 20 minutes on each of five successive days.

Protein-free Broth (Uschinsky). Sodium chloride 5.0 g., calcium chloride 0.1 g., magnesium sulphate 0.2 g., potassium acid phosphate 2.0 g., potassium aspartate 3.0 g., ammonium lactate 6.0 g., distilled water to 1000 ml. Dissolve and add 30 ml. of glycerin. Tube, and sterilise by heating at 100° for 20 minutes on each of three successive days.

Saponin Broth (Blood Cultures). Nutrient broth with addition of 2% sodium citrate. Add white saponin (B.D.H.) 0.1%. Sterilise by steaming for half an hour on each of three successive days. To each 10 ml. add 2 ml. of blood to be cultured. (Final concentration of saponin 0.08%.)

Tryptic Digest Broth (Hartley Broth). Ox heart or lean beef (free from fat and minced) 1500 g., tap water 2500 ml. Mix together and heat in the steam steriliser until a temperature of 80° is reached. Then add sodium carbonate (anhydrous) 0.8% solution (cold) 2500 ml. Cool to 45° and add pancreatic extract 50 ml. Incubate at 37° for six hours, the liquid being frequently stirred. When digestion is completed add 40 ml. of pure strong hydrochloric acid, steam for 30 minutes and then filter. Adjust with N/1 NaOH to neutrality with phenolphthalein. Bottle and steam for 20 minutes. For storage add 0.25% of chloroform and when cool keep in cool place. When required for use steam for 20 minutes to remove chloroform, filter and adjust to the required reaction. **Pancreatic Extract** (Coles and Curlow) is prepared from fresh pig pancreas (fat-free and minced) 500 g., distilled water 1500 ml., dehydrated alcohol 500 ml. Shake the mixture and allow to stand at room temperature for 3 days shaking occasionally. Strain through muslin and filter through Chardin paper. Add sufficient strong hydrochloric acid to the filtrate to produce 0.1%. Allow to stand for several days and filter. This extract keeps indefinitely if stoppered bottles are used. If used at once the hydrochloric acid need not be added as its use is to retard the slow deterioration of the trypsin. The following may be substituted for 50 ml. of the above pancreatic extract, **Liquor Trypsin Co. (A & H)** 30 ml., or **Bacto-Trypsin (Digestive Ferments Co.)** 30 ml.

For other media described in the text consult the Index.

STERILISATION

Sterilisation, or the preparation of sterile material, is the removal or killing of living organisms. Removal may be accomplished by filtration through a bacterial filter, and killing may be done by dry heat, moist heat, disinfectants or a combination of these methods. The following are important factors affecting the viability of bacteria:—Temperature, the presence or absence of water, the presence or absence of food material such as proteins and carbohydrates, the hydrogen ion concentration of the medium, the presence of substances having a bactericidal action.

Temperature. The optimum temperature for the growth of pathogenic organisms is 37°. Non-sporing organisms are all killed by heating at 60° for 1 hour in the presence of water (i.e., moist heat). Some succumb at much lower temperatures, e.g., gonococci, which are killed at 47° in a few minutes. A possible exception is *Streptococcus faecalis* which is said to require a slightly higher temperature than 60°. The usual infection in preparing sterile material and solutions is from the hands and is generally a staphylococcus (such as *S. albus*) which may be killed by heating at 80° for 10 minutes. This short heating process is recommended as a safety precaution after packing and

sealing the product in its final container, provided the active substance is stable at that temperature.

Sporing organisms require a higher temperature than non-sporing. No spores will survive a temperature of 115° of moist heat for 30 minutes (using an autoclave) or a temperature of 150° of dry heat for 1 hour.

Bacterial growth usually ceases below 10° but it appears to be impossible to kill bacteria by low temperatures. Bacteria exposed to the temperature of evaporation of liquid oxygen (−190°) and liquid hydrogen (−252°) are not killed.

The following temperatures are of interest.

| | |
|------------|---|
| 0° to 10° | Most bacteria will survive for long periods without multiplying. Organisms will remain alive longer at this temperature than at room temperature. |
| 12° to 15° | Average room temperature. |
| 22° | Optimum temperature for growing moulds and saprophytes. |
| 37° | Blood heat. Optimum temperature for growing pathogenic organisms. |
| 42° to 45° | Optimum temperature for enzymic reactions. |
| 65° | Proteins begin to coagulate, and all are coagulated at 80°. |
| 100° | Moist heat. 5 minutes kills vegetative bacteria. |
| 115° | Moist heat for 30 minutes will ensure sterility. |
| 150° | Minimum temperature for 1 hour to ensure sterilisation with dry heat. |
| 160° | Highest dry temperature which will not char cotton fabric. |

Hydrogen Ion Concentration. Bacteria have an optimum pH for growth, usually about pH 7·0, whilst on either side of this optimum point there are limits beyond which they fail to survive. These vary with different bacteria. Thus:—

| | Acid Limit pH | Optimum pH | Alkaline Limit pH |
|-------------------------------|---------------------|---------------|-------------------------|
| <i>B. coli</i> | 4·4 | 6·0 to 7·0 | 7·8 |
| <i>Vibrio cholerae</i> | 5·6 | 6·2 to 8·0 | 9·6 |
| <i>B. paratyphosum</i> | 4·0 | 6·2 to 7·2 | 9·6 |
| <i>Cl. welchii</i> | 4·7 | — | 11·1 |
| <i>Chr. prodigiosum</i> | 5·0 | 6·0 to 7·0 | 8·0 |
| <i>Streptococcus viridans</i> | 7·3 | 7·6 to 7·8 | 8·3 |
| <i>B. typhosum</i> | 4·0 | 6·2 to 7·2 | 8·7 |
| <i>Staphylococcus aureus</i> | 5·6 | 7·2 to 7·6 | 8·1 |

The correct pH is therefore an important factor in the medium used for promoting growth. Thus, in the preparation of culture media, as in the anaerobic and aerobic broth used in testing for sterility and the standard Rideal-Walker broth used for testing the bactericidal values of disinfectants, it is important to make a careful adjustment to pH 7.6 to secure optimum conditions for growth. Conversely an unsuitable pH has an important bearing on the ease of sterilisation. Many pharmaceutical solutions have a pH at or near the value unfavourable to infecting organisms. Davis (*Quart. J. Pharm.*, 1934, 381) has shown that the following, even after gross infection with *B. mycoides*, *B. subtilis*, dust from straw packing, *Cl. sporogenes* and a soil filtrate, are sterilised by steaming for 60 minutes (the pH of the solutions undoubtedly contributes to the ease of sterilisation): Atropine sulphate 0.12%, codeine phosphate 5%, caffeine and sodium benzoate 25%, normal saline, dextrose 5% and 30%, homatropine hydrobromide 0.6%, procaine hydrochloride 2%, pilocarpine nitrate 3%, sodium thiosulphate 12%, strychnine hydrochloride 0.75%, peptone 5%, sodium salicylate 30%, hexamine 20%, amylocaine hydrochloride 5%, soluble barbitone 10%, calcium chloride 5%, morphine hydrochloride 2.5%, morphine tartrate 2.5%, phenazone 40%.

The majority of these solutions proved to be sterile after 30 minutes' steaming, but this period is unreliable and should be extended to 1 hour.

Methods of Sterilisation

The 4th Addendum revises the methods of sterilisation as given in the British Pharmacopœia, 1932. *Glass Vessels and Containers* should be freed from grease, preferably by washing with soap or with an aqueous solution of sodium cetyl sulphate or similar product, rinsing with distilled water and sterilising by heating either (a) in a hot air oven at 150° for one hour or (b) autoclaving at 115° for 30 minutes. When glass apparatus is sterilised in a hot air oven, care should be taken that both the heating up and cooling down processes are not too sudden. This applies particularly to new glassware. Rubber in any form will not stand this treatment; rubber goods should be boiled or autoclaved. The oven may be electrically or gas heated with thermo-controls, but it can be improvised. A shelf or false bottom, perforated or otherwise arranged for air circulation, must separate the articles from the bottom plate upon which the flame plays. It is advisable to prepare a supply of sterilised apparatus and to keep it in a special cupboard which should be reasonably dust-free. Flasks and bottles for such stock should be either plugged with non-absorbent wool or capped with paper and tied with twine before placing in the oven. Other apparatus should be wrapped in paper (newspaper is quite suitable) or cloth. All wrappings must remain on until the apparatus is required for use. Alternatively, the apparatus (including rubber tubing, bungs and surgical rubber gloves) may be sterilised by autoclaving at 115°

for 30 minutes. The apparatus should be capped or wrapped before autoclaving, and, except rubber material, immediately dried (whilst still wrapped) in an air oven at about 60° to 70°. Mortars and pestles can be sterilised by well washing with soapy water, rinsing with sterilised water and drying by flaming with a bunsen burner. Pestles with wooden handles will not stand 150°, since the resin-cement fixing the head may melt.

Sterilisation of Aqueous Solutions. The 4th Addendum to the *B.P.* permits (a) autoclaving, (b) heating with a bactericide, and (c) filtration through a bacteria-proof filter.

Autoclaving. The official process of autoclaving is intended for aqueous solutions or preparations, which are placed in their final sealed containers and heated in steam at 115° to 116° for 30 minutes. A single exposure to these conditions in an autoclave is sufficient to destroy all bacteria and spores provided that the temperature is maintained for that length of time.

Volumes of solution over 100 ml. are required to be heated for a longer period than 30 minutes. Jackson (*Pharm. J.*, 1934, 181) gives the following periods as necessary to bring the respective volumes to 115°, distilled water in flat-bottomed flasks being used.

| | | | | | |
|-----------------------|----|-----|-----|-----|------|
| Vol. of liquid in ml. | 50 | 100 | 250 | 500 | 1000 |
| Time in minutes | 11 | 15 | 18 | 23 | 28 |

In the preparation of injection of sodium chloride and acacia, the *B.P.* requires a preliminary autoclaving at 121° to 122° for one hour, owing to the poor heat conductivity of the solution, and the usually gross infection of the gum. The second autoclaving process is probably unnecessary if reasonable aseptic precautions are taken, steaming being sufficient. It is an advantage to avoid re-autoclaving as the gum tends to give a precipitate again. Watson (*Pharm. J.*, i/1937, 527) gives details of a method for clarifying gum saline under aseptic conditions. Autoclaving is of special value only when used for aqueous solutions; when applied to the treatment of oily solutions it is only equivalent to heating them in a hot air oven at the same temperature.

Autoclaves are usually fitted with pressure gauges which register lbs. pressure in excess of atmospheric. It is to be regretted that all are not also fitted with a thermometer as the gauges are liable to become inaccurate with use. The only true check is when gauge and thermometer register the correct corresponding pressure and temperature. The following shows the temperatures corresponding to the various pressures, thus giving saturated steam.

| Pressure (in excess of atmospheric pressure) | Temperature |
|--|-------------|
| 5 lb. | 109° |
| 10 lb. | 115.5° |
| 15 lb. | 121° |
| 20 lb. | 126° |
| 25 lb. | 130.5° |

These pressures and temperatures *will not coincide* when

(a) air remains mixed with the pressure steam;

(b) insufficient water is placed in the autoclave with the result that the steam becomes superheated and *unsaturated* (under these conditions, aqueous solutions in unsealed containers concentration will occur).

Precautions in the Use of an Autoclave

(1) See that sufficient water is placed in the autoclave.

(2) Do not wire ordinary rubber caps on to vaccine bottles containing the solution. The caps will burst during autoclaving. It is advisable to insert a thin copper or silver wire between the cap and bottle to provide an air vent. The cap may be wired on after cooling in the autoclave. This procedure, however, is not in accordance with official instructions to seal the container prior to autoclaving. Berry (*Pharm. J.*, i/1937, 397) gives details of a special rubber vaccine cap which may be wired on and will then withstand autoclave pressures above 10 lb., and also (*Pharm. J.*, i/1938, 627) details of a special vaccine bottle which does not require the cap to be wired on.

(3) Allow steam to issue freely from the steam cock for several minutes before shutting down in order to drive the air out of the autoclave. An aqueous solution in an unsealed container should be placed in the autoclave when the water in the latter is boiling, otherwise there may be an appreciable concentration occurring during the process of driving out the air. This precaution is unnecessary when sealed containers, such as ampoules, are treated.

(4) Time the immersion from the point when the gauge reaches the correct pressure.

(5) At the end of the period allow the pressure to come gradually to zero before opening up. If a sudden reduction in pressure is made by opening the steam cock, solutions in unsealed containers will boil away vigorously. The autoclave should not remain closed after zero pressure is reached, as further cooling produces a negative pressure which may strain the gauge. Moreover a negative pressure is quickly produced which may cause the warm solution to boil and burst the cap if the precautions described above have not been taken.

(6) Rubber capped vaccine bottles should be allowed to cool in the autoclave. If wired whilst hot, the caps tend to sink on cooling.

An autoclave suitable for small scale work is the Sankey. For an account of this see Greenish and Holder (*Pharm. J.*, i/1932, 355).

Heating with a Bactericide. This process is intended for preparations which cannot withstand the thermal conditions of the autoclave. Either 0.2% *w/v* of chlorocresol or 0.002% *w/v* of phenylmercuric nitrate is added. When the volume does not exceed 30 ml. the solution is then heated in the final sealed container at 98° to 100° for 30 minutes. For larger volumes the

time of heating must be extended so that the contents are maintained at 98° to 100° for 30 minutes. *Intravenous injections* must not be prepared by this method when a single dose exceeds 15 ml. *The method must not be used for intrathecal or intracisternal injections.*

Oily Solutions and Suspensions. These should be distributed in their final containers, which are then either permanently or temporarily sealed so as to exclude bacteria. When the volume does not exceed 30 ml. they are then heated at 150° for one hour. For larger volumes the heating must continue for a longer period so that the whole of the solution or suspension is maintained at 150° for one hour. The temporarily sealed containers should then be sealed. If the medicaments will not withstand this heat treatment, the preparation may be made by aseptic methods, using oil which has been sterilised by heating at 150° for one hour. The preparation must then be transferred to previously sterilised containers and sealed.

Fats, Waxes, Soft and Liquid Paraffins may also be sterilised by this method. Wool fat should always be treated in the same way since from the nature of its source there is the possibility of contamination with anthrax and tetanus. Care should be taken not to overheat vegetable oils such as olive oil, since partial decomposition may occur. It is advisable and convenient to allow oils to filter through filter paper whilst sterilising in the oven. The efficiency of the method is confirmed by Coulthard and by O'Brien and Parish (*Quart. J. Pharm.*, 1935, 90, 94). Coulthard stresses the importance of ensuring that the oil reaches 150°, and the fact that it is unwise to rely on oven-temperature only. The thermometer should dip into a control quantity of oil in the oven so that the temperature indicated would be that of the oil. Samples of olive and almond oils so treated showed no significant deterioration, no increase in the acid value being noticeable.

Dry Chemicals and Powders. For these dry heat is best, preferably 150° for 1 hour, provided the physical characters (decomposition, m.p., volatilisation, loss of water of crystallisation, etc.) of the substance permit. The chief problem is to obtain effective heat penetration owing to the poor conductivity of such material, hence powders should always be spread out in a thin layer in the oven. Kaolin (like wool fat) should always be suspect as from the nature of its source it may contain tetanus spores. It is best sterilised by being spread out in a thin layer on paper in the oven and heated at 160° to 170° for one hour. It may then be tipped quickly into a sterilised, wide-mouthed, stoppered bottle, care being taken to avoid undue exposure to re-infection whilst doing so. Zinc oxide may be similarly heated. (As a guide to temperature, paper and cotton wool turn yellow at about 165°). Tawell advises sterilising boric acid by prolonged heating at 98° in a carefully regulated oven. It is liable to undergo change if heated much above 100°.

Tyndallisation. This process is now no longer official, it having been deleted by the 4th Addendum. It consists in heating the solutions in sealed containers at 80° for 1 hour on 3 successive days, and is applicable to substances which are unstable at higher temperatures, such as salts of cocaine, atropine, etc. The process is based upon the theory that the vegetative forms of bacteria will be readily killed at 80° but that spores will survive. These, however, will change to the vegetative form during the resting period and will be killed in the subsequent reheatings. The process is usually too long for practical purposes and has, moreover, been seriously criticised as being unreliable. The success of the process depends upon whether or not the spores actually do develop into the vegetative form during the resting period. Such development during this period will only occur if optimum conditions prevail, such as the presence of nutrient material, a suitable pH and the absence of any substance having a bacteriostatic action. Davis (*Quart. J. Pharm.*, 1940, 14) shows that it is an unreliable method for solutions of most medicaments. It still remains a useful method for the sterilisation of culture media which it is undesirable to autoclave. O'Brien and Parish (*Quart. J. Pharm.*, 1935, 94) record the results of tests on the attempted sterilisation of oils by tyndallisation, using olive and almond oils and liquid paraffin infected with a spore-containing mixture of earth, hay and faeces, and they condemn the method as unreliable. This again is undoubtedly due to the oily medium being unsuitable for development of the vegetative form.

Filtration through a Bacteria-proof Filter. For sterilisation by filtration the British Pharmacopœia requires control by tests for sterility. This is a wise precaution, since filtration is a skilled operation and demands good aseptic technique. It is probably the best sterilisation process for large scale work and where efficient control is available. For isolated small scale work it is probably too complicated for general use.

The process is the only one for substances such as insulin which are easily inactivated on heating. It can, however, be applied generally except where adsorption of the medicament may occur, as in the case of indigo carmine and methylene blue. The adsorption of substances of this type is apparent by loss of colour, but the possible adsorption of other medicaments by the various types of filters requires investigation. **Bacterial filters** for pharmaceutical use are available in several types. The filter material varies and may consist of—

(a) diatomaceous material (the German Berkefeld, the Mandler and the Salador filters. These are soft filters and must be carefully treated).

(b) porous porcelain (the Pasteur-Chamberland and the Doulton. These are hard filters and are very strong).

(c) asbestos pads (Seitz and Sterilmats).

(d) sintered glass (the Jena 5 auf 3 filter).

(e) collodion membrane (Cella membranes).

German Berkefeld Filters are made in three grades determined by the rate of flow of water: W (wenig), N (normale), V (viel). The finest grade (W) is suitable for pharmaceutical use. Because of the diatomaceous composition of the filter, adsorption of medicament from a solution may be appreciable and the first runnings should be rejected.

Mandler Filters were introduced as an American substitute for the German Berkefeld filter. The filters are graded according to the lbs. pressure necessary to force air through against water. Those graded as 15 lbs. are suitable for pharmaceutical use, and are very reliable filters. Being of a diatomaceous composition, the same precautions should be taken as regards adsorption as with German Berkefeld filters.

Saludor Filters are British filters and very suitable for pharmaceutical use.

Pasteur-Chamberland Filters are made of unglazed porous porcelain and are graded as L1, L2, L3, L4, L5, etc., L1 being only a clarifying filter. L3 will stop ordinary bacteria such as *B. coli*, but for general reliability in pharmaceutical work, it is advisable to use an L5. Finer grades than this are usually too slow for general use. Some adsorption of medicament is possible and should be guarded against.

Doulton Filters of unglazed porous porcelain are similar to the Pasteur-Chamberland and have a pore diameter equal to an L5 type. Doulton filters of this type are quite suitable for pharmaceutical use. Doulton "Dialas" filters are not suitable for pharmaceutical use as they are too coarse and are highly adsorbent.

For details of apparatus using the Doulton porous porcelain filter see Sykes, *Pharm. J.*, i/1934, 521.

Seitz Filters are made of an asbestos composition in various grades of which the *E. K. Special Bacterial* is the type for pharmaceutical use. They consist of pads which are held in special filter holders. The assembled filter can be worked with both positive pressure using a bicycle pump (Manteufel model), or negative pressure using a water pump (Uhlenhuth model).

Sterilmats are British asbestos composition pads. Grade S.B. is recommended for pharmaceutical sterilisation processes.

Positive pressure is preferable since any leakage is outwards with less risk of air-borne contamination. New pads may be used for each filtration. They cannot be cleaned, but their price is such that their use is very economical. Whilst for general use these filters are efficient, they are open to serious objections for pharmaceutical use. They tend to yield alkali to solutions and may cause the precipitation of free alkaloid from solutions of alkaloidal salts. Solutions of medicaments which are sensitive to alkali, such as insulin, pituitary extracts, adrenaline, apomorphine, etc., should not be filtered through a Seitz filter as inactivation may occur. Another serious disadvantage is the traces of fibre which the pads yield, and considerable difficulty may be experienced in obtaining a filtrate free from such contamination. This is a serious objection for intravenous injections. It may be overcome by attaching a small sintered glass filter to the metal outlet of the Seitz filter. This effectively removes the fibres. For details of such a filtering apparatus see White, *Pharm. J.*, ii/1934, 355, and Berry, *Pharm. J.*, i/1936, 96.

Seitz and Sterilmats pads depend for their efficiency, to a certain extent, on the swelling of the fibres with water, and where such swelling does not occur, as with strong alcohol, their efficiency may be lowered. Adsorption of the medicament may also be very appreciable.

Sintered Glass Filters, 5 auf 3 type. They are made of two layers of sintered or frittered glass, a very fine layer of No. 5 superimposed on a coarser No. 3 supporting layer, the two being fused into a glass funnel. These filters vary considerably in pore size and each filter is issued by the manufacturers with a certificate giving the maximum and average pore values. Those having a maximum pore value of 2.5μ or less are reliable bacterial filters for pharmaceutical use. Those having a value of less than 1.5μ will generally be found to be too slow. They are excellent filters for small scale work as they can be readily cleaned with strong sulphuric acid containing a little potassium nitrate, which treatment does not affect the pore size. They are not suitable for large scale work as the filtering area is too small. The adsorption properties are much less than the porous porcelain or diatomaceous filters. For details of

sintered glass 5 auf 3 filters and a comparison of pore size of various filters see Berry, *Pharm. J.*, i/1936, 61 and 96.

Collodion Membrane Filters (Cella filters). These filters consist of very thin circular membranes of nitrocellulose and are supplied graded according to pore diameter. Those having pore diameters between 0.5 and 0.75μ are suitable for pharmaceutical use. The membranes are placed on a porous base in a special filter holder and a new membrane is used for each filtration operation. The membranes must not be allowed to dry and are usually stored in 2% formalin solution or in Nipagin solution. According to Thomann they will not withstand autoclaving but should be steamed at 100° for one hour after assembly in the apparatus (which has been previously autoclaved). For details, see Thomann.

Adsorption effects are practically absent. The filters have a very high pore density and consequently a rapid flow. They are particularly suitable for small scale work.

Cleaning Bacterial Filters. The process of filtration, using a "candle" filter, is best done from the outside to the inside. All impurities will then be deposited on the outside, and can easily be removed. After each operation filter candles should be cleaned by gently scrubbing the outside with a fine brush in a stream of running water. They should then be washed by forcing water through in the reverse direction to that used in filtration. With long usage filters may be gradually choked with an accumulation of organic matter, chiefly dead bacteria, and drastic treatment may be necessary. Unmounted porcelain candles may be heated to a bright red in a muffle furnace, being afterwards allowed to cool in the furnace. This latter precaution is important, since too sudden cooling may result in the development of small cracks and loss of efficiency. The filters should then be well washed by passing a stream of water through them. The Berkefeld, Mandler and Salador filters may be freed from heavy protein contamination by immersion in an alkaline (pH 8.5) trypsin solution at 37° overnight and then well washing with water.

It should be understood that bacterial filters may allow organisms to pass when the time of filtration is prolonged, since they tend to grow through the filter bed. For an account of this see Horrocks, *Brit. med. J.*, i/1901, 60. For accounts of sterilisation by filtration see Hunwicke, *Pharm. J.*, i/1932, 350; Coulthard, *Pharm. J.*, i/1933, 286 and 338; Berry, *Pharm. J.*, ii/1937, 267 and 294.

Aseptic Precautions. It is an advantage to reduce the risk of infection of solutions for injection to a minimum during the process of preparation and sterilisation. It is especially important to take these precautions when conducting sterilisation by filtration, or when broth media or preparations containing them are being handled.

Hands should have nails carefully attended, and kept short and clean, being well scrubbed with soap and water. Liquor Chloroxylenolis B.P. Add. VI. undiluted should then be rubbed in for five minutes. Scrubbing with soap and water alone has no bactericidal value and will not sterilise the hands. It may in actual fact increase the number of bacteria by bringing them to the surface from the lower layers of skin.

Clothing. White overalls washed at frequent intervals should be used and should be kept in a cupboard in the laboratory. This precaution lessens the danger of contamination from clothing exposed to street dust.

Air. It is important to realise that bacteria are chiefly dust-borne, and any precaution which results in the removal of dust from the atmosphere will render the latter reasonably free from organisms. Dust may be removed by (a) filtration or (b) steaming. The room should be designed so as to avoid unnecessary ledges on which dust can collect, and the walls, benches, floors, etc., should be such as to permit of frequent washing with an antiseptic. When filtration is relied upon, the air is usually drawn in through a cotton wool filter or through an oil filter, and a positive pressure is maintained in the room so that when a window or door is opened air escapes outwards and dust cannot enter. The deposition of dust by steaming can be carried out by creating a slight cloud of steam from the ceiling in the room by attaching rubber tubing to a kettle or boiler. As the moisture settles it will carry the dust down. The following are results of steaming carried out in the serum preparation laboratory of a London hospital, counts being made on agar plates exposed for a definite time.

| | Count |
|---|-------|
| Before steaming | 15 |
| During steaming | 45 |
| $\frac{1}{2}$ hour after steaming | 2 |
| 2 hours after steaming with 2 persons working | 3 |

Bacteria are not killed by this method but only carried down to the floor or bench.

Restriction of number of workers. The laboratory should be restricted to those who are actual operators, as every additional person means extra contamination.

For small scale work when a special room is not available, and the work has to be done in the ordinary dispensary, an excellent method is to use a case with sliding doors, similar to a fume cupboard (without the fume vent), the interior of which can be washed and is not subject to the same dust deposition as the remainder of the room. Such precautions are advisable if filtration sterilisation is employed. For similar apparatus see Gunn, *Pharm. J.*, i/1935, 327.

The Dispensing of Sterile Parenteral Injections

When several doses are prescribed in one container, the solution or preparation must contain a bacteriostatic agent in such a concentration as will prevent the growth of micro-organisms. The following are suitable: phenol 0.5%, cresol 0.3%, chlorbutol 0.5%, chlorocresol 0.1%, phenylmercuric nitrate 0.001%, sodium ethylmercurithiosalicylate (Merthiolate) 0.01%. The last mentioned is only stable in neutral or alkaline solution. Since all these

substances may react with rubber, the rubber caps used for closing the containers must be boiled in several changes of water and then either boiled for 30 minutes, or stored for not less than 48 hours in a solution containing the same bacteriostatic agent and in the same concentration as that used in preparing the injection. There are several medicaments which themselves have a pronounced bacteriostatic action. It is unnecessary to add another bacteriostatic agent. Examples of these medicaments are iodophthalein, mersalyl, leptazol, quinine dihydrochloride and acid sulphate. *Intrathecal and intracisternal injections must be dispensed only in containers each of which contains a single dose. This means restricting the containers to ampoules only.*

Storage of Medicaments. A simple precaution which can and should be taken is to keep all medicaments likely to be used for the preparation of injections in bottles filled with a type of stopper which prevents dust falling between the stopper and the neck, and also to reserve such stock for this special purpose by keeping it in a separate cupboard reasonably dust-free. Such stock should not be used for ordinary dispensing purposes, such as the preparation of mixtures, pills and powders. Most medicaments when stored in ordinary stoppered dispensing rounds can quickly become infected in the daily routine of dusting, since dust will be driven between the neck and stopper.

Sterilised Water. The *B.P. Add. I* describes the method for the preparation of sterilised water and the *U.S.P. XII* includes a special test for pyrogens in sterilised water used for parental injections (*see pp. 79-81*).

Lees and Levvy (*Brit. med. J.*, i/1940, 430) have described an emergency method for the preparation of pyrogen-free water, omitting the distillation process altogether. Tap water is boiled with powdered activated charcoal, which adsorbs the pyrogens. Biological tests on rabbits are used for the estimation of pyrogen content. The method is successful with Cardiff municipal water (Berry, *Pharm. J.*, i/1941, 146, 100). For a description of the preparation of non-pyrogenic saline solutions using activated charcoal *see* Todd, *Pharm. J.*, i/1941, 258. The activated charcoal should not yield soluble matter, particularly calcium salts, to water.

Sterilisation of Surgical Dressings

Much attention has been paid during recent years to methods for the sterilisation of surgical dressings, following a report by Fildes of a heavy tetanus spore infection in cellulose wadding dressings and by Pulvertaft (*Brit. med. J.*, i/1937, 441) of the occurrence of tetanus and welchii spores in sanitary pads and even in so-called "sterile" accouchement sets. Cellulose wadding appears to be very prone to infection by pathogenic anaerobic spores. A committee set up in 1939 by the Royal College of Obstetricians and Gynaecologists investigated the problem and

reported (*J. Obstet. Gynaec.*, 1941, 394) that brown cotton-wool is nearly always heavily infected and white cotton-wool, though less heavily infected, is seldom free from spore-bearing anaerobes. The method of manufacture (weaving, etc.) of fabrics tends to produce very bacteriologically dirty material, which is not easy to sterilise. In view of the universal use of dressings, they should be viewed with no less suspicion than catgut. The report of the committee dealing with the use of the dressings-autoclave stresses the importance of complete permeation of the dressings by moist heat maintained at the correct temperature for a proper time. In order to effect this, air must be removed by vacuum before the steam is turned on. The dressings must be packed loosely and never pressed down. Cotton-wool in any quantity should be unrolled and dealt with in loose layers. *Rubber gloves* should be packed flat, with each glove between a layer of gauze, and sterilised at a temperature of 105° for 15 minutes. The vacuum produced before introducing the steam should be equivalent to a negative pressure of 15–20 inches of mercury and this should be held for 5 minutes before the steam is turned on. When the pressure gauge reads 20 lbs. the steam valve should be adjusted and this pressure held for 20 minutes, then a negative pressure of 15–20 inches of mercury kept for 15 minutes. This will conclude the sterilisation, but the dressings will be wet. In order to produce dry dressings, hot dry air should be admitted very slowly, taking at least 10 minutes in the process, and it should be drawn through a thick layer of sterile cotton-wool. According to Savage (*Quart. J. Pharm.*, 1936, 366) dressings contained in packets need not be unsealed, as steam will penetrate almost immediately. Savage (*Quart. J. Pharm.*, 1937, 451) shows that there is no evidence that mixtures of air and steam are less effective for sterilising than steam alone, provided the spores are exposed to the saturated atmosphere and are really at the temperature they are supposed to be. Steam retains its sterilising power, even when superheated by 5° or 15° or more, depending on its initial temperature. Possibly this is due to the fact that the essential feature of steam sterilisation is the equilibrium between the aqueous solution constituting the bacterial cell and the steam atmosphere, which equilibrium is attained when the steam is saturated with respect to the solution, not when it is saturated with respect to pure water.

Heat Penetration. Sterilisation can only be ensured if the steam penetrates the interior of the dressings and the temperature is maintained for the requisite time. It is important, therefore, to have some device which will register these conditions. A thermo-couple placed in the interior of a typical dressings packet and registering outside the autoclave is one such device. Savage (*Quart. J. Pharm.*, 1940, 237) recommends an earth spore packet placed in the interior of the dressing and afterwards tested for sterility. Witness tubes or tubes termoins consisting of a powdered mixture of some substance melting at the sterilising temperature

and a dye have been suggested. Tubes of this mixture are inserted in a dressing and permanently change colour when the sterilising temperature is reached. The following are suitable:—Terpin hydrate with 0.1% methylene blue melts at 115° and changes to an even blue colour, acetanilide (113°) and methylene blue and sulphur (115°). It should be noted, however, that these powders will only register temperature, and not the duration of heating which is so important.

Tests for Sterility of Surgical Dressings. The technique of testing surgical dressings for sterility requires greater skill than testing a liquid, because it is necessary to remove the dressing from its wrapper without infecting the former. Rigid aseptic precautions must be taken and the operation conducted under a screen, preferably in a laboratory designed for aseptic work. Savage (*Quart. J. Pharm.*, 1940, 237) gives a description of the process and recommends a searing iron for burning open the wrapper. He maintains that it is not possible to exclude laboratory infection entirely. The dressing is transferred aseptically to sterile jars and sufficient melted "sloppy" nutrient agar medium poured over to cover it. The agar medium contains 0.4% of agar and 2% of dextrose. Incubation should be maintained at 37° for 5 days.

DISINFECTANTS

The bactericidal or bacteriostatic efficiency of disinfectants is a subject which is assuming greater importance every year, for apart from their use in hygiene and surgery, their use in the field of curative medicine is steadily increasing. Our present knowledge of these substances is incomplete; the value of many published experiments cannot be assessed because of the omission of important details of procedure, yet an exact knowledge is very essential because of the important rôle they are called upon to play and the great dependence placed upon them.

In devising tests for bactericidal action the following factors must be taken into account:

(a) **The concentration of the disinfectant.** It does not follow that by doubling the concentration, the time of disinfection is halved.

(b) **The time during which the disinfectant is in contact with the test organism.** This will vary with other factors such as temperature and concentration, but it must be recognised that some substances can exert a high bactericidal power if sufficient time be given. Thus mercuric chloride is capable of acting as a bactericide even in high dilution (1 in 400,000) if sufficient time is allowed for the action. Similarly, acriflavine requires a long period to demonstrate bactericidal activity.

(c) **The temperature at which the test is conducted.** The rapidity of bactericidal action will increase with rise of temperature but again will vary with the substance. Thus the rapidity of

action of mercuric chloride is increased threefold by a rise of temperature of 10° , whereas that of phenol is increased five to sevenfold.

(d) **The organism chosen as the test organism.** Most disinfectants vary in their bactericidal power towards different organisms. Thus, phenol is much more active against streptococci than against staphylococci. It is essential, therefore, to select one particular organism if comparisons are to be made. One organism, however, may exist as different strains of appreciably different resistance towards one particular disinfectant. Hence it is further necessary to specify the strain of the test organism, and in order to get complete concordance the organism should also be subcultured under standard conditions through several generations. *B. typhosum* has been chosen for the Standard Rideal-Walker test because of the greater reliability of its strains, the ease with which it grows on agar and also because it is an organism which it is desired to destroy in practice. It forms a fairly uniform suspension in broth culture.

(e) **The presence of other substances having a modifying action.** The bactericidal value of a disinfectant may be considerably modified by the presence of other substances. Thus, sodium chloride present in the phenol solution will increase the value, whilst alcohol and glycerin will lower it. Koch has shown that phenol when dissolved in vegetable oils has practically no value. McMaster (*J. inf. Dis.*, 24, 378) has shown that mineral oils such as liquid paraffin do not produce this effect; the efficiency of phenol in liquid paraffin is nearly as great as in aqueous solution. The effect of the presence of protein matter such as serum, blood and faecal matter is of great importance in assessing the value of a disinfectant. Substances which react with protein matter usually have a much lower bactericidal value in its presence than in aqueous solution. Thus, the value of mercuric chloride is reduced by 90% in the presence of serum. Formaldehyde and solutions of hypochlorites, e.g., Dakin's solutions or compounds of a similar type, such as chloramine, react very quickly with protein and their bactericidal value drops correspondingly. Iodine will kill streptococci in a few minutes at a dilution of 1 in 120,000 in distilled water, but the addition of 5% of blood requires a concentration of 1 in 1000, and 50% of blood 1 in 200, to prevent actual growth (Fleming, *Proc. roy. Soc.*, Ser. B, 1924, 171). The value of crystal violet and brilliant green against staphylococci suffers a big reduction in the presence of serum. Phenols and cresols, however, are very little affected by the presence of protein and the bacteriostatic values of acriflavine and related compounds are unaffected by the presence of serum. Urea, uric acid, fats, alkalis and acids will also affect the action of disinfectants. These reactions in protein media are of great importance in selecting a disinfectant for a particular use. The uses can be very variable, for disinfectants may be required for (1) the sterilisation of apparatus and surgical instruments; (2) the disinfection of

excreta, drains, utensils, closet pans, soiled linen and the washing of walls and furniture; (3) the preservation of vaccines and sera against possible bacterial contamination in the presence of a considerable amount of soluble protein matter; (4) the disinfection of wounds.

It follows from these considerations that no one disinfectant can be satisfactory for every purpose and it is essential to use them in an intelligent manner by studying the type of work required from them. It also follows that there can be no one standard test which will give the bactericidal value for a particular disinfectant for all the conditions in which it may be applied.

Standard Methods of Testing Disinfectants

There are two standard tests in use: (a) the Rideal-Walker, which tests the disinfectant in aqueous solution only, and (b) the Chick-Martin, which tests in the presence of protein matter (yeast).

The Standard Rideal-Walker Test. Was first published in 1903 but several modifications of it have been introduced, not only by the authors but by other workers. Since disinfectants are sold on this test it is essential that the same standard procedure should be followed. The method laid down by the British Standards Institution and prepared under the supervision of the Chemical Divisional Council (*B.S.S.* No. 541—1934) is now the accepted standard. Its chief application is for coal-tar disinfectants. It should be clearly understood that the test merely indicates the value of the disinfectant compared with phenol under the conditions of the test and does not enable an opinion to be expressed as to its value under practical conditions of use. Extended experience has shown that this standardised technique, if carefully followed, will give concordant results in the hands of competent workers. The strictest adherence to every detail is essential, and the tests should be performed under reasonably dust-free conditions in the laboratory. All the factors which can cause a variation in aqueous solution are standardised.

Apparatus. The specification includes descriptions of the inoculating loop, temperature of the incubator, pipettes, medication tubes, broth tubes and measuring cylinders, and attention is drawn to the fact that all apparatus must be scrupulously clean and sterile immediately before use.

Reagents. The reagents used are (1) Standard Rideal-Walker broth. (2) A suitable culture of *Bacterium typhosum* obtained from The Curator, National Collection of Type Cultures, Lister Institute, Chelsea Gardens, S.W.1. (3) Standard phenol (carbolic acid) having a crystallising point of not less than 40.5°C.

Standard Rideal-Walker Broth

20 g. of peptone (Allen & Hanburys' "Eueptone") rubbed to a cream with water is added slowly to a boiling solution of 20 g. of Lab-Lemco in 750 ml. of water; 10 g. of sodium chloride is added and the liquid boiled for thirty minutes, cooled, and water added to one litre. An aliquot portion is titrated at 37° with N/10 sodium hydroxide until neutral to phenolphthalein, and the calculated quantity of N/1 sodium hydroxide is then added very slowly to the remainder to render it neutral to phenolphthalein at 37°. The mixture is heated to the boiling-point and filtered while hot. By the addition of N/1 hydrochloric acid the reaction of the solution is adjusted.

to pH 7.6, using phenol red as indicator. The broth is sterilised by heating in an autoclave, filtered when cold, distributed into tubes each containing 5 ml., and again autoclaved. The final reaction of the broth is between pH 7.3 and pH 7.5.

Method. The technique of the method is described fully in the specification and an example given of the method of calculating the coefficient. By this method the R.W. coefficient is obtained by dividing that dilution of the disinfectant which permits growth in 2½ and 5 minutes but no growth thereafter, by that dilution of carbolic acid (1 : 95, 1 : 100, 1 : 105, 1 : 110, or 1 : 115) which permits growth in 2½ and 5 minutes but no growth thereafter.

There have been many suggested modifications of the Rideal-Walker type of test. Details of a large number of experiments on common medicinal substances with one modification, using *B. coli* as the test organism (known as the "Lancet" method) conducted by W. H. Martindale will be found in the 19th Edition of this volume, p. 266 *et seq.*

The Standard Chick-Martin Test. The original Chick-Martin Test was devised at the Lister Institute and first described in *J. Hyg.*, 1908, 8, 655. It was designed to determine the value of disinfectants in the presence of organic matter. As disinfectants have frequently to act in the presence of large amounts of organic matter this feature of the test is of considerable practical importance. The organic matter in the original test was dried fæces, but Garrod (*J. Hyg.*, 1934, 34, 322, and 1935, 35, 219) criticised it as unsuitable and recommended yeast instead. This recommendation was adopted in the Standard Chick-Martin Test as published by the British Standards Institution (*B.S.S.*, No. 808—1938). Whilst the new test gives more reproducible results than the old one, it should be understood that the results have reference to yeast only and that the presence of other types of protein matter may give different results. The test does, however, give some idea as to whether a disinfectant is liable to have its value reduced in the presence of organic matter.

The details laid down in the Standard Test must be strictly observed.

The Test Organism is the same strain of *B. typhosum* as for the Rideal-Walker Test.

The Standard Broth is prepared by dissolving Lab-Lemco 10, peptone (Allen and Hanburys "Eupeptone") 10, and sodium chloride 5, in distilled water 1000, boiling for 30 minutes cooling and adjusting to 1000 with freshly boiled and cooled distilled water. The broth is then adjusted to pH 7.6, filled into tubes and sterilised by autoclaving at 15 lbs. pressure per sq. inch for 20 minutes. The final product should have a pH lying between 7.3 and 7.5.

Yeast Suspension. A 5% dry weight suspension of yeast in distilled water is used, adjusted to pH 7. A suitable yeast is D.C.L. special moist yeast marketed as "Yeast for B.S.I. C/M tests" by The Distillers Co., Ltd., Cheese Lane, Bristol.

Method. A mixture of 48 ml. of a standard yeast suspension and 2 ml. of *B. typhosum* culture is prepared, and 2.5 ml. of this mixture is added to each prepared dilution in distilled water of the disinfectant under test and to standard dilutions of phenol. Subcultures into standard broth are then made at 30-minute intervals. All these operations are done with solutions whose temperature is controlled by a water-bath maintained at $20^{\circ} \pm 0.5^{\circ}$.

Calculation of Phenol Coefficient. The phenol coefficient is calculated by dividing the mean of the highest concentration of phenol permitting growth in the broth cultures and the lowest concentration of phenol showing absence of growth in both cultures by the corresponding mean concentration of the

disinfectant. The technique of the test and method of calculating the result is described fully in the specification.

The Specific Action of Disinfectants. Many disinfectants exhibit variable bactericidal values when tested against different organisms. A knowledge of this specific action is of great importance, since it governs the choice of the disinfectant likely to be most effective against a particular pathogenic organism. This specific action is very apparent in the organic dye series of disinfectants and in many cases it appears to vary according to the staining properties of the organisms. Thus, with a mixture of different organisms, it is possible to select a particular disinfectant which will kill off one organism in the mixture and allow the others to grow profusely. Churchman (*J. exp. Med.*, 1912, 221) showed that most of the gram-positive bacteria are very susceptible to gentian violet, crystal violet, malachite green, aniline violet, and safranin, but that the gram-negative bacteria will grow well in concentrations of these dyes twenty times as strong. Later, he showed that by substituting acid fuchsine or flavine for the gentian violet type the result could almost be reversed and the gram-negative bacteria inhibited while allowing the gram-positive cocci to grow.

The following concentrations are necessary to inhibit the growth of *Staphylococcus aureus* and *B. coli*.

| | <i>Staph. aureus</i> | | <i>B. coli</i> | |
|----------------------------|-----------------------------|----------------|-----------------------------|----------------|
| | Conc. in 0.7% peptone water | Conc. in serum | Conc. in 0.7% peptone water | Conc. in serum |
| Crystal violet | 1 : 4,000,000 | 1 : 400,000 | 1 : 8000 | 1 : 8000 |
| Malachite green | 1 : 10,000,000 | 1 : 40,000 | 1 : 20,000 | 1 : 1000 |
| Brilliant green (sulphate) | 1 : 10,000,000 | 1 : 30,000 | 1 : 130,000 | 1 : 3500 |
| Brilliant green (oxalate) | 1 : 10,000,000 | 1 : 100,000 | 1 : 200,000 | 1 : 3500 |
| Flavine | 1 : 20,000 | 1 : 200,000 | 1 : 1300 | 1 : 100,000 |
| Phenol | 1 : 250 | 1 : 250 | 1 : 500 | 1 : 500 |
| Chloramine-T | 1 : 2000 | 1 : 250 | 1 : 2000 | 1 : 250 |
| Mercuric chloride | 1 : 1,000,000 | 1 : 10,000 | 1 : 1,000,000 | 1 : 10,000 |

General Disinfectants for Hospital and Domestic Use

For the disinfection of utensils, fabrics, rubber sheets, benches, floors, drains, excreta, etc. Generally speaking, disinfectants for these purposes must:—

- be quickly effective against a wide range of organisms,
- retain their germicidal activity in the presence of much protein matter, such as blood or faecal matter,
- be cheap and without disagreeable odour.

These considerations result in the use mainly of phenolic disinfectants for these purposes. The commercial disinfectants made from phenols are of the types (a) *Lysol*, (b) *Black fluids* such as Jeyes Fluid, (c) *White fluids* such as Izal.

Lysol or *Liquor Cresolis Saponatus* is official under these names in the British Pharmacopœia. It contains 50% *v/v* of cresol in a saponaceous solvent. Lysol is effective against a wide range of organisms and is probably the most suitable cheap disinfectant for general hospital purposes, because (a) it contains soap whose detergent action is very useful for removing grease, (b) it gives clear dilutions with water, (c) it is bactericidal against a wide range of organisms, including both gram-positive and gram-negative, (d) it is less reduced in efficiency in the presence of organic matter than most other disinfectants. In any concentration in which it is an effective germicide it is caustic to the skin, and is, therefore, unsuitable for skin disinfection. It does not attack rubber and when strong solutions of lysol are being employed rubber gloves should be worn.

To be safe, a disinfectant must be used for sufficient time and at an adequate concentration. The following are suitable concentrations and times of contact for the varied uses of lysol:—

| Use | Dilution and Time | Conditions |
|--|--|---|
| Scalpels, needles, instrument forceps | Undiluted for at least one minute | Rinse in sterile water prior to use. |
| Baths, bowls, bins, dishes, etc. } if can- not be boiled | Undiluted for at least one minute | Rub thoroughly over the surface, using mop or rag in rubber-gloved hand. Afterwards wash well with tap water. |
| Rubber sheets, sorbo-rubber mattresses | $\frac{1}{2}$ fl. oz. to 1 pint of water | Sponge with mop soaked in this solution and afterwards rinse thoroughly with tap water. |
| Unboilable fabrics, jars for instrument forceps | $\frac{1}{2}$ fl. oz. to 1 pint of water for at least 1 hour | — |
| Septic towels | 1 in 3 for one hour | — |
| Washing walls and floors | 1 fl. oz. to 1 pint of water | Use in a bucket with a mop. |

Black fluids and **white fluids** are preparations from tar acid fractions containing phenols of higher boiling ranges than cresol. They are used for large scale disinfection when a cheap product is wanted. Black fluids are solutions in soap and when poured into water give stable white emulsions. White fluids are concentrated emulsions prepared with soaps, sulphonated oils, glue, gelatin, casein or dextrin. White fluids are usually better than black fluids in the presence of hard water. In the presence of organic matter their germicidal values are very similar. They are less caustic and less poisonous than lysol, but possess a more penetrating and persistent odour. Their phenol coefficients vary

with different organisms, but they are usually good general disinfectants and in the dilutions recommended by reputable makers, there is a good margin of safety.

The following results were obtained with a typical black fluid:—

| Organism | R.W. Phenol Coefficient |
|-----------------------------|-------------------------|
| <i>Staphylococcus</i> | 3.5 |
| <i>Ps. pyocyanea</i> | 4.5 |
| <i>B. dysenteriae</i> | 10.0 |
| <i>B. coli</i> | 10.0 |
| <i>B. typhosum</i> | 11.0 |
| <i>B. pestis</i> | 34.0 |

These black and white fluids may be used as follows:—

| Use | Dilution | Conditions |
|------------------|--|---|
| Linen. . . | White fluid with Chick-Martin coefficient of at least 3.0. 1 fl. oz. to 1 pail of water. | Leave overnight. |
| Bed-pans .. | White or black fluid (if heat sterilisation not possible). 1 fl. oz. to 1 quart of water. | Thorough mechanical cleansing by flushing and scouring followed by the disinfectant for one hour. |
| Excreta .. | White and black fluid. (Chick-Martin coefficient of at least 3.0.) 1 fl. oz. to 1 quart of water. | — |
| Sinks, drains. . | Black fluid. Flush with one pail of hot water containing a handful of washing soda and then rinse with 1 fl. oz. to 1 pail of soapy water. | — |
| Floors, walls .. | For local pollution. Black or white fluid, 4 fl. oz. to 1 pint. | — |

Black Fluids.—The British Disinfectant Manufacturers' Association state that the following British proprietary disinfectants are of the black fluid type:—

Antifect; Bactocene; Coctas; Disolite; Exenol Superior; Hycol; Hygenol Fluid Disinfectant; Ialine Special; Jeyes Fluid; Kilcrobe 10/12 Black; Killgerm 10/12 Black; Lawes; Metro; Monsanto Black Disinfectant No. 4; Sanitas-Bactox; Septol; Special Creitas; Cooper's Standard Fluid; Vitalin No. 99.

White Fluids.—The British Disinfectant Manufacturers' Association state that the following British Proprietary disinfectants are of the white fluid type:—

Baxol X; Botazone No. 1; Exenol D.A. White; Ialine No. 8; Izal; Jeyes White Cyllin; Kilcrobe 18/20 White; Killgerm 18/20 White; Lawes L.W. 4; Monsanto White Disinfectant Fluid No. 1; Sanitas-okol; Sal-Hycol; Star White; Sterilite; Supelrin White; White Bactocene; White Kerol; White Septol; Wrights Vetersol; Voxsan M.A.F. Disinfectant Fluid.

Chlorinated Lime is a cheap, effective disinfectant and deodoriser for drains.

Soaps and Detergents. Aqueous solutions of soaps vary in their germicidal action which is never very rapid. The chief value of soaps lies in their detergent and wetting action whereby micro-organisms can be easily washed away from surfaces rather than killed on them. They have little action on bacterial spores. Those possessing the greatest activity against non-sporing organisms are coconut oil, palm kernel oil and resin acid soaps. Ordinary soaps in aqueous solution owe their detergent action to the structure of their anions and such germicidal action as they possess is due to these. There are other "anionic" detergents available which are not soaps, such as sodium cetyl and lauryl sulphates (Dreft and Sulphonated Lorol are commercial preparations containing these substances). These compounds are excellent detergents and like ordinary soap, owe their action to the anion *cetyl* or *lauryl*. They possess some bactericidal action, but mainly against gram-negative organisms. There are, however, "cationic" detergents in which the cation is responsible for detergent action. Several of these compounds possess marked germicidal power extending over a much wider range of organisms than "anionic" detergents and are effective against both gram-positive and gram-negative organisms. Anionic and cationic detergents are incompatible with each other and when solutions of them are mixed together, detergentcy and bactericidal action are lost.

Types of these "cationic" detergent-disinfectants are:—

Cetyltrimethylammonium bromide, $C_{18}H_{33}(CH_3)_3NBr$, was introduced as a proprietary preparation—Ctab. This compound will ionise giving $[C_{18}H_{33}(CH_3)_3N]^+$ as the cation, and Br^- as the anion. In aqueous solutions it is a powerful detergent, frothing readily, but the solution has a fish-like odour. According to Barnes (*Lancet*, i/1942, 242, 531) it shows great promise as a skin and wound disinfectant and probably as a general disinfectant. Its bactericidal value is reduced in the presence of serum and it is inactivated in the presence of soap.

Alkylbenzyltrimethyl ammonium chloride, $RC_6H_5CH_2(CH_3)_3NCl$, where R is a mixture of radicals such as lauryl and cetyl, etc., obtained from coconut oil. It was introduced as the proprietary preparation Zephirol or Zephiran. Favourable results have been reported as a skin and wound disinfectant. Its value is reduced in the presence of serum but not so far as to inactivate it (Dunn, *Proc. Soc. exp. Biol.*, N.Y., 1936, 35, 427). It loses much activity in the presence of soap.

Excretmental Disinfectants. Disinfectants used for the sterilisation of excreta, sputum, etc., must be substances which retain their germicidal power in the presence of excess of protein matter. They are usually required to act fairly quickly and of necessity must be reasonably cheap. The various black and white coal tar fluids and Lysol are generally used for faeces. Sputum cups should preferably be sterilised by autoclaving.

According to Adams (*Tubercle*, 1938, 19, 208) the following are of little value for sterilising sputum containing the tubercle bacillus:—Phenol in aqueous solution, soap solutions of chloroxylenol, and alkylbenzyl-dimethyl-ammonium chloride solutions. Lysol, however, in a dilution of 25% was very efficacious, giving complete sterility after 30 minutes exposure.

Economy in the Use of Disinfectants

The Therapeutic Requirements Committee of the General Medical Council, as a war-time measure, issued an appeal for the curtailment of the use of phenol, cresol and mercury compounds as disinfectants. All these substances are wanted for other very important purposes.

Recommendations. In general, bactericides should not be used when disinfection by heat is possible. Heat treatment is more efficient and reliable. Infected matter should be removed, with all due precautions by scrubbing and scouring, where practicable.

Storage of Instruments and Suture Needles. These may be kept in Liquor Boracis et Formaldehydi N.W.F. and rinsed with sterile water before use. This method should not be used for hypodermic needles.

Rubber Tubing. This should be sterilised by boiling at the time of using. If it is thought necessary to keep sterile tubing ready, it can be autoclaved and kept in a sterile towel.

Rubber Sheeting. Scrubbing with soap and water is all that is necessary, unless the sheeting is known to be contaminated in an exceptional way. Heavily contaminated sheeting should be scrubbed and then boiled or autoclaved; if this is impossible it should be scrubbed and sponged over with rag soaked in lysol ($\frac{1}{4}$ fl. oz. to 1 pint of water) and then rinsed thoroughly with tap water.

Linen. This should be boiled if possible, otherwise it should be soaked overnight in a dilution of a "white fluid" disinfectant having a Chick-Martin coefficient not lower than 3.0 (1 fl. oz. to 1 pail of water is usually strong enough with a good "white fluid").

Baths. Lysol, undiluted, should be rubbed thoroughly over the surface, using a mop or rag in a gloved hand, for at least one minute. The bath should then be washed well with tap water. Other surgical utensils—e.g., arm-baths, bowls, dishes, etc., should be boiled whenever possible, otherwise they should be disinfected as for baths and washed finally with sterile water.

Bed-pans. If sterilisation by heat is impracticable, thorough mechanical cleansing by flushing and scouring, followed by the use of a "white" or "black" fluid disinfectant is recommended.

Excreta. When direct disinfection of excreta is necessary a "black" or "white" fluid, having a Chick-Martin coefficient not lower than 3.0, should be used in accordance with the instructions issued by the manufacturer (1 fl. oz. to 2 quarts of water is usually advisable).

Sinks, Drains. Thorough flushing is most important. Chlorinated lime is a cheap and efficient disinfectant. Washing soda, 1 oz. to 1 quart of hot water, may be used for cleaning glazed earthenware. A "black fluid," 1 fl. oz. to 1 pail of water, may be used as a deodorant.

Floors. As a deodorant, a "black fluid," $\frac{1}{4}$ fl. oz. to 1 pail of soapy water may be used. For local pollution, a "white fluid" or a "black fluid," $\frac{1}{4}$ fl. oz. to 1 pint, should be used.

Phenol Antiseptics. For most purposes "black" and "white" fluids, if of the same efficiency, as judged by the Chick-Martin coefficient, are equally suitable. The tendency of "black" fluids to cause staining, however, may be a disadvantage in some cases.

Antiseptics in Wounds

It is now generally agreed that no antiseptic has so far been produced which will actually kill bacteria in a wound without at

the same time damaging tissue and killing leucocytes. The important role of antiseptics in wounds should be bacteriostatic; inhibiting the growth of infective organisms whilst permitting phagocytosis to proceed. This is all that is necessary; phagocytosis and the natural antiseptic action of the enzyme lysozyme which is present in most body tissues, constitute the main defences against bacterial infection. Any antiseptic used should in no way interfere with these two processes. Fleming has shown that in certain concentrations some antiseptics in the presence of blood may actually produce conditions more favourable to bacterial growth than in blood without the antiseptic because the leucocytes are killed. Maclean (*Pharm. J.*, ii/1932, 287) gives instances in which the bactericidal value of lysozyme is destroyed by certain antiseptics such as iodine and hypochlorites. Modern surgical treatment of wounds stresses as most important, prompt excision of the dead portions, which if allowed to remain may provide a good culture media for bacteria. For cleansing the wound of dirt, Trueta (*Brit. med. J.*, i/1942, 616) states that no antiseptic is known to-day equal to soap as a means of dealing with contamination in a wound and that sodium coconut oil soap and sodium ricinoleate are the most effective of the soaps for this purpose. After debridement and cleansing, the wound may be treated with a suitable antiseptic. Failing prompt surgical treatment, an antiseptic may be of great value if applied without delay, particularly if the wound is not deep. When infection is deep seated, antiseptic treatment is useless.

The following are in use as antiseptics for wounds:—

Acridine Derivatives, such as acriflavine, proflavine, acrirubrine.

Acriflavine (*syn.* Flavine) was introduced by Browning as an antiseptic for wound treatment. It was claimed that it retained its activity in the presence of blood or serum and that it had no deleterious effect on the tissues or leucocytes (*Brit. med. J.*, ii/1917, 70). It is used as a wound dressing either as a solution in isotonic saline 1 in 1000 or as Emulsion of Acriflavine, B.P.C. It has, however, been much criticised; Fleming (*Lancet*, ii/1917, 341) claimed that it was destructive to leucocytes and that this action was far in excess of its bactericidal action. This contention seems to be borne out by the fact that wounds, after prolonged treatment with acriflavine, tend to become sluggish in healing. It has recently been criticised by Russell and Falconer (*Brit. med. J.*, ii/1941, 378) who state that acriflavine solution, 1 in 1000, damages brain tissue, causing intense necrosis and hæmorrhage, whilst proflavine sulphate (proflavine) and Acrirubrine (2:7-diaminoacridine hydrochloride) in the same concentration were not more injurious to the brain tissue than normal saline. These latter are accordingly recommended for the prophylactic treatment of brain wounds. The solutions should be buffered at pH 6.2. There appears to be no reason for using acriflavine when proflavine is available. According to Albert *et al.* (*Brit. J. exp. Path.*, 1938, 19, 41) acrirubrine possesses a much lower mammalian toxicity than proflavine, whilst the two are approximately equal in bacteriostatic activity.

All the acridine antiseptics showed marked specificity towards certain organisms, generally being most effective against streptococci, less towards staphylococci and quite ineffective against *Ps. pyocyanea*. All solutions of these compounds should be protected from light and it is important to note that cotton dressing adsorbs these dyes and immobilises their antiseptic action. Emulsion of Acriflavine, B.P.C., has been criticised by Garrod as a useless preparation because the acriflavine is in aqueous solution as the disperse phase in a water-in-oil emulsion and therefore cannot make contact with the wound. This preparation is, however, largely used as a first aid dressing.

Penicillin. An antibacterial substance obtained from cultures of *Penicillium notatum* by Fleming in 1929, promises to become an important wound antiseptic as well as a potent therapeutic agent. It is developed by the mould in ordinary nutrient broth after 8-10 days incubation at room temperature and recently Florey and others (*Lancet*, ii/1941, 177) have isolated it in solid but impure form. This product is remarkably active, inhibiting the growth of *Streptococcus pyogenes*, *Staphylococcus aureus*, anthrax, meningococcus and certain other bacteria in a dilution of 1 in 1,000,000 or more, gram-positive organisms being more sensitive to it than gram-negative. It has no observable toxic effect on leucocytes in concentrations which are powerfully bacteriostatic and its potency is not affected by blood or serum. It is not affected by pus, peptones or other products of cell breakdown nor by large numbers of dead or living organisms, which latter characters distinguish it from the sulphonamide compounds. It is innocuous to fibroblasts in tissue cultures and appears to be without toxic effect to man or animals when administered intravenously. It is unstable in acid or alkaline solution and should be used in neutral medium, although it will not retain its activity even then for more than one month. Excellent reports of its efficacy in indolent wounds have been published. According to Fleming it compares with the sulphonamide compounds as follows:—

| | Smallest concentrations showing no growth | |
|----------------------|---|------------------------------|
| | <i>Streptococcus pyogenes</i> | <i>Staphylococcus aureus</i> |
| Penicillin | 1 in 800,000 | 1 in 320,000 |
| Sulphathiazole | 1 in 200,000 | 1 in 80,000 |
| Sulphapyridine.. .. | 1 in 50,000 | 1 in 10,000 |

The fact that the impure penicillin only affects leucocytes in a concentration of 1 in 250 while a concentration of 1 in 2,000,000 shows some inhibition of growth of the common pyogenic cocci makes it likely that it will be extremely valuable for wound treatment.

Sulphonamide Compounds. Although not directly bactericidal, these compounds appear to stimulate phagocytosis and bring about a selective bactericidal action. They have been extensively tried in pyogenic wounds. They are inhibited to some extent by organic matter, particularly if the products of extensive tissue breakdown are present, but even in the presence of pus, their local application reduces the streptococci, staphylococci, coliforms and spore-bearing anaerobes in wounds. According to Miles (*Lancet*, ii/1941, 507), there is every justification for their early employment in the treatment of wounds by direct application as a powder, for even if they fail to destroy the bacteria, they may prevent their multiplication and thereby permit the delays in full surgical treatment. Oral administration of these drugs has proved ineffective against gas-gangrene organisms, but local application with the solid drug has been most effective. *Cl. Welchii* infections have been most successfully treated, *Cl. septicus* less and *Cl. oedematiens* least. Sulphathiazole has proved superior to sulphanilamide and sulphapyridine against these three organisms and on balance against streptococci, pneumococci and staphylococci. Sulphathiazole appears to owe some of its local efficacy to its moderate solubility, which lying midway between that of sulphanilamide and the more insoluble sulphapyridine ensures that it goes into solution slowly but in effective concentrations. It will be carried away in the circulation, and Hawking (*Brit. med. J.*, i/1941, 511) has suggested that the local concentration should be supplemented by oral administration of the drug.

Certain conditions may hinder or completely abolish the antiseptic effect of sulphonamide drugs. The number of bacteria present has a depressing influence, as also have a large number of dead bacteria. The same hindering effect has been shown with peptones and pus fluid. In the latter medium, sulphapyridine even in a dilution of 1 in 64 fails to inhibit streptococci (Fleming, *Pharm. J.*, ii/1940, 162). Sulphonamide drugs, therefore, may not be very effective in highly septic wounds, but could be used with advantage after surgical treatment of the wound if secondary infection is feared.

Hypochlorites. Hypochlorites, mainly as sodium hypochlorite solution, came into use as wound antiseptics during the 1914–1918 war as a result of the work of Carrel and Dakin. They depend for their bactericidal action on their available chlorine, probably as hypochlorous acid, which acts rapidly on bacteria and on most protein matter. Because of this latter reaction, hypochlorite solutions soon lose their activity in wounds, and it is necessary continually to supply fresh solution in order to maintain it. *Dakin's Solution* is an aqueous solution of sodium hypochlorite containing 0.5% w/v of available chlorine and buffered with boric acid to a pH of about 9.7. This is a reasonably stable preparation and not sufficiently alkaline to be irritating in the wound. It is generally used as a 2% solution for continuous irrigation. This procedure is necessary because the available chlorine is rapidly exhausted by interaction with the proteins of the tissues. Its value as a wound antiseptic has been adversely criticised by Fleming (*Brit. med. J.*, i/1940, 631) who states that its only important action is a lymphagocytic one, causing an increase in the rate of transudation of fluid into the wound and tending to drain the infected area. Recently hypochlorite solutions prepared by electrolytic action have been claimed as superior to Dakin's solution in wound treatment, but the validity of such claims is doubtful. For a method of preparation of electrolytic hypochlorite solution see Sykes, *Public Pharmacist*, 1942, 2, 90.

Eusol is a solution of calcium hypochlorite prepared by digesting bleaching powder in water, filtering and dissolving boric acid in the filtrate. Whilst it is an active bactericidal solution it is unstable and can, with advantage, be replaced by Dakin's Solution.

Chloramine (Chloramine-T). A 2% aqueous solution is used as a wound antiseptic, is non-irritating and very stable. It is inactivated less quickly by protein than Dakin's solution but also acts more rapidly.

Azochloramid (N-N-dichloroazodicarbonamide). A proprietary substance, reacting more slowly than chloramine-T with protein matter (Schmelkes and Hornung, *J. Bact.*, 1935, 29, 323). It is claimed to be non-irritating and also to be effective in blood serum and therefore useful as a wound dressing. It is used as 1 in 500 solution in glycerin triacetate or 1 in 1600 and 1 in 3300 solutions in isotonic buffered saline solutions. According to Russell and Falconer the triacetin solution should not be used in brain surgery.

Iodine. Whilst a very good disinfectant for intact skin it may be unreliable as a wound antiseptic, particularly in the presence of much blood. Fleming (*Proc. roy. Soc., Ser. B.*, 1924, 171) has shown that in the presence of 50% of blood, a concentration of 1 in 200 is required to inhibit streptococci, although in aqueous media without blood, 1 in 120,000 will kill these organisms.

Hydrogen Peroxide owes its effect in wounds largely to the mechanical action of the liberated oxygen in cleansing suppurating wounds by disturbing the deposits of pus. According to Russell and Falconer it should never be used in brain surgery as it causes fragmentation with consequent hæmorrhage of the cerebral tissues.

Chlorophenols. The substitution of chlorine in the benzene ring of a phenol considerably increases the bactericidal effect towards certain organisms. Trichlorophenol, chlorocresol (*p*-chloro-*m*-cresol) and chloroxylenol (*p*-chloro-*m*-xylenol) have all been recommended as wound antiseptics. Their bactericidal and bacteriostatic values are reduced by protein matter, more so than is the case with phenol and cresol. *Chloroxylenol* is used as a solution in soap in many proprietary preparations and as *Liquor Chloroxylenolis B.P. Add. VI*. Its main use is for the sterilisation of intact skin for its bactericidal value is considerably reduced by the presence of blood, or serum. For the treatment of wounds it is used undiluted and probably retains sufficient bacteriostatic value to be useful. It is very effective against hæmolytic streptococci but much less effective against staphylococci and still less against *Ps. pyocyanea*. Colebrook (*Bull. war Med.*, Vol. II, No. 2, 1941, p. 73) recommends for the suture line at the first dressings of a clean wound as follows:—If any washing is required use a 20% solution of chloroxylenol. If no washing is required dust with sulphanilamide powder as a precaution against hospital infection and cover with a dry dressing.

Bismuth and Iodoform Paste (B.I.P.P.). This has been used in wound treatment, the paste being applied as a thin covering over the wounded surface which is then dressed in the usual way. Excellent results in the treatment of

acute osteitis.—Shaw, *Lancet*, i/1933, 250. Tropical ulcer well treated.—Kerby, *Lancet*, i/1932, 237. Garrod (*Pharm. J.*, i/1935, 323) states that it has no *in vitro* action on either streptococci or staphylococci.

Zinc and Iodoform Paste (Z.I.P.P.) has been recommended as an improvement on *B.I.P.P.* Excellent results reported on over 300 wounds, including open fractures. No cases of gas-gangrene, iodoform poisoning, dermatitis or maggot infestation. The replacement of the zinc oxide with zinc peroxide was not satisfactory.—Connell, *Lancet*, ii/1940, 22.

Sterilisation of the Skin

Proper disinfection of the unbroken skin is of real importance to the surgeon, midwife and all engaged in the handling of sterile material. The skin is no longer regarded as a mere mechanical barrier against bacterial invasion, but possesses a biological mechanism whereby it can free itself from organisms which do not constitute its own natural bacterial flora. These latter consist mainly of staphylococci and diphtheroids and no washing or scrubbing with soap and water will remove them. Colebrook (*J. Obstet. Gynaec. Brit. Emp.*, 1933, 40, 966) investigated the problem of killing hæmolytic streptococci on the skin in gynæcological practice. He concludes that ordinary yellow household soap is a more efficient killing agent for streptococci than toilet soaps and soft soap, and the effect is not due to the pH of the soap. There is, however, no margin of safety in merely washing the hands with such soap. After washing the hands with soap for a minute and then soaking for 3 minutes in any of the following solutions, sterilisation was not accomplished, for staphylococci could still be cultivated from the skin:—

| | |
|-------------------------|--------------------|
| Mercuric Chloride | 1 in 1000 |
| Lysol | 1 in 160 |
| Formalin | 3% in 5% Glycerin. |

Complete elimination of organisms was obtained by using:—

| |
|---|
| Crystal Violet, 1 in 200, in 30 minutes |
| Brilliant Green, 1 in 200, in 30 minutes |
| Lysol, 1 in 160, in 5 minutes |
| Chloramine-T, 1 in 100, in 3 minutes |
| Aqueous Iodine, 1 in 50 with 3% KI, in 1 minute |
| Dettol Paste in 2 minutes |
| Undiluted Dettol in 1½ minutes. |

Iodine, chloramine-T and Dettol (or Liquor Chloroxylenolis, *B.P. Add. VI*) stand out clearly as efficient skin sterilisers because of the rapidity and completeness of effect. Rapidity of effect is essential as it is inconvenient for the operator to have to wait for long periods.

Lysol, in effective concentration, is too irritating to the skin and therein lies the danger that the operator may be tempted to dilute beyond the effective concentration. **Iodine** is unsuitable for continuous use, mainly because of the risk of toxic dermatitis. It is, however, eminently suitable for preoperative use. A distinction should be drawn between the bactericidal value of iodine on the skin and its use in wounds, for in the latter its value is greatly reduced. It requires a strength of 1 in 120 in 50% of blood to prevent the growth of streptococci, whilst 1 in 120,000 in water will kill these organisms in 3 minutes.

Liquor Tinctorium, B.P.C., contains the same proportion of crystal violet and brilliant green as the solutions mentioned above and apparently is not of outstanding merit.

Iodine and Chloroxylenol Solutions, according to Colebrook, give protection to the skin from subsequent infection with hæmolytic streptococci for several hours, whilst lysol 1 in 160 and mercuric chloride 1 in 1000 only for 30 minutes. For general use as skin disinfectants, therefore, preparations of chloroxylenol would appear to be the most suitable.

Alcohol has an extensive use as a skin disinfectant. Its optimum strength for this purpose is 70%, but its action is unreliable, particularly if the skin is greasy.

Chloramine-T in 1% solution has come into use as a reliable substitute for iodine for swabbing the skin prior to an injection.

Carbol-fuchsin as a paint containing 5% of phenol is an excellent bactericide and fungicide for skin infection. Excellent results with ringworm.—Pillsbury, *Arch. Derm. Syph.*, N.Y., 1942, 45, 61.

Mercury Compounds. Mercuric chloride and mercuric iodide have long had a reputation for efficiency in the sterilisation of the skin. Their bactericidal action, however, is *very slow* and their bacteriostatic action very pronounced. This latter action may interfere with tests for sterility and has given false values to the bactericidal efficiency of mercury compounds. They are, therefore, unreliable as skin disinfectants. The newer organic mercury compounds, such as phenylmercuric nitrate and mercurochrome are similarly unreliable.

Sterilisation of Atmospheres

The problem of the sterilisation of atmospheres presents itself in two forms:—

- (a) to sterilise the atmosphere and the surface of the contents of a room in the absence of individuals living in it. This can be done fairly effectively with vapours of formaldehyde, sulphur dioxide, phenol or cresol, but no individual could withstand such an atmosphere for even a few seconds;
- (b) to sterilise an atmosphere, and to maintain its sterility without rendering it toxic to human beings or animals.

Lister, in 1871, attempted to produce a sterile atmosphere in the operating theatre with his carbolic spray, but later abandoned it, because he recognised that it was not effective except in such a high concentration as would make it intolerable to breathe. Since Lister's time the urge to find a reliable method has increased, and much work has been done and data obtained, but until quite recently no really practical solution to the problem was apparent.

There are two main types of air infection; in one the bacteria are dry, and are generally associated with dust particles. In this case the flora can be very mixed, and consists mainly of non-pathogenic aerobes, but may contain such pathogens as hæmolytic streptococci, and anaerobes such as tetanus and the gas-gangrene organisms. The other type of infection is in the form of droplets enclosing organisms expelled from the respiratory tracts of human beings and animals. The air in sanatoria has been shown to contain living tubercle bacilli, and that of fever wards to have the causative streptococcal organisms present. The problem has suddenly become of national importance because of crowded air-raid shelters, places which generally were not designed for long habitation and a dense population.

Aerosol Mists.

Spraying the air in these places with solutions of bactericides in the form of coarse spray is a useless method, but such solutions in the form of aerosols or mists (spray in which the droplets are approximately of colloidal dimensions) may show marked bactericidal power. Surprising results have been obtained. Thus many powerful bactericides usually credited with high bactericidal values such as amyl-*m*-cresol, *o*-phenylphenol, lysol and chloroxylenol proved very disappointing as aerosols, being either ineffective or too irritating for use. On the other hand, some substances, such as resorcinol, which normally are not regarded as being very potent, proved very efficient when the solutions containing them were properly formulated. A most effective preparation is 10% of hexylresorcinol in propylene glycol and containing 0.05% of sulphonated lorol (Twort, *J. Hyg., Camb.*, 1940, 40, 253). This solution is too expensive for large-scale work, but a cheap and very effective substitute is sodium hypochlorite. In dealing with the problem of air-raid shelters, Andrewes (*Lancet*, ii/1940, 770) finds that 5 ml. of a 0.5 to 1.0% solution sprayed into the air will rapidly kill 95% of hæmolytic streptococci, influenza virus and other organisms suspended in the air in a room of about 1000 cu. ft. capacity in 10 to 15 minutes. This represents a concentration of 1 part of hypochlorous acid gas in 1-2 million parts of air. If properly atomised this quantity of solution should last in that size room for 30 minutes, but if loss occurs through ventilation, it may be necessary to atomise continuously. In this concentration hypochlorite mists are not unpleasant and have deodorising properties which are useful. A mist causing irritation to the conjunctiva or respiratory tract should be regarded as too strong. Hypochlorite mists will, however, attack metal and should not be used in the vicinity of electrical equipment. They also tend to become ineffective when the air is very dry or if they are mixed with the vapours of phenols and cresols.

Organisms enclosed in droplets are very susceptible to bactericidal mists, whilst bacteria on dust or on surfaces are much less susceptible. The design of the spraying machine is most important. All operate on the perfume spray principle with a baffle plate to catch and retain the larger inert droplets. Hypochlorite solutions cannot be used in metal sprayers. Andrewes (*Lancet*, ii/1940, 770) and a circular issued by the National Institute for Medical Research, give details and dimensions of a simple box spray which can be operated by power or by a foot pump for use with hypochlorite solutions. Whilst the main use of bactericidal mists is against droplet infection, it is obviously impossible to kill the organisms so instantaneously as to prevent infection by what may be termed projectile inoculation, whereby the uninfected person receives infection in a space of a second due to a cough or a sneeze. The same conditions apply to a chlorinated swimming bath. This can only be prevented by masking the infected persons. An aerosol mist can, however, reduce a floating droplet population of pathogenic organisms and materially minimise the risks of infection.

Dust.

Bacteria associated with dust are usually unaffected by aerosol mists. Dust on floors can be prevented from rising by treating the surface once a month with spindle oil (crude liquid paraffin). This method has been recommended for hospital wards and for bedclothes (including blankets), (Thomas and Ende, *Brit. med. J.*, i/1941, 953). Tubercle bacilli, hæmolytic streptococci and diphtheria bacilli are amongst those which may survive in dust.

Ultra-violet Light.

Ultra-violet light is very effective against bacteria in liquid droplets and on very fine particles (Andrewes, *Lancet*, ii/1940, 770). When on dust particles, however, bacteria appear to be highly resistant. The results under practical conditions, such as in hospital wards and operating theatres, have been variable. It is necessary to have some form of air filter in the duct leading to the ultra-violet lamp with a forced draught arrangement. It has been possible to obtain air almost or wholly free from bacteria at the exit of the duct. Such a system has been used in a number of trials in operating theatres. It does not appear to be a practical method for large air-raid shelters because of the cost of installation and maintenance.

RADIUM

Radium ($Ra = 226.0$) is a disintegration product of uranium and is therefore found in all uranium minerals, such as pitchblende, which contains about 80% of uranium oxide, carnotite, potassium uranyl vanadate, etc. The ratio of radium to uranium in the minerals is about one to three million. Owing to the minute proportion present, the separation is a lengthy and expensive process. If the mineral contains no barium, a small quantity of a soluble barium salt is added to it, and the barium is then separated by the usual chemical methods. The whole of the radium associates itself with the barium, which it closely resembles. The radium is then separated from the barium by repeated fractional crystallisations of the chlorides or bromides, the radium halogen salts being less soluble in water and dilute mineral acids than the corresponding barium salts. Its chief compounds are the hydroxide, $Ra(OH)_2$, the halides, $RaCl_2$, $RaCl_2 \cdot 2H_2O$, $RaBr_2$, $RaBr_2 \cdot 2H_2O$, the nitrate, $Ra(NO_3)_2$, the sulphate, $RaSO_4$, and the carbonate, $RaCO_3$. Both in chemical properties and appearance, all these compounds closely resemble the analogous barium salts, the most stable being the sulphate.

The radioactive elements possess, in addition to the normal physical and chemical properties corresponding to their position in the periodic group, the property of radioactivity, that is, of emitting radiations, the atom spontaneously disintegrating until a stable atom (an atom which is not radioactive) is formed, the final result of disintegration being certain of the isotopes of lead.

Radium was first prepared by Madame Curie and M. A. Debierne (1910) in the pure basic condition by electrolysis of a solution of a radium salt, using a mercury cathode. The 25th anniversary of the discovery of radium by M. Pierre Curie and Mme. Curie was celebrated at the Sorbonne on December 26th, 1923. A Bill passed by the French Government granted Mme. Curie a pension of 40,000 francs a year.

Distribution. Although from time to time various pitchblende and other uranium-containing ores have been mentioned as being worth operating for radium both in this country and abroad, it is stated that about 95% of the world's output was obtained from the uranium ore deposits in the Belgian Congo (Haut Katanga). Recent discoveries in Canada of rich sources of radium are, however, altering the situation. The pitchblende deposits in the Great Bear Lake district in the North-West Territories, which constitute the Empire's most valuable source of radium and uranium, have been worked for a number of years. The ore, which also carries important quantities of silver and copper, is concentrated locally and sent by aeroplane to a refinery at Port Hope, Ontario, where radium bromide and sulphate, uranium nitrate and oxides, polonium, silver-copper concentrate and silver-cobalt-copper-nickel concentrates are produced. The radium produced in 1937 and 1938 amounted to 23,770 mg. and 70,000 mg. respectively whilst the uranium compounds produced in 1937 totalled 546,000 lb. Owing to war conditions the production of ore from

Great Bear Lake has been suspended, but the Port Hope refinery is continuing with the stocks of ore already in hand.

Commerce. The radium salt now chiefly in request is the sulphate which is most suitable for the preparation of radium applicators, the chloride and bromide being preferable for emanation purposes. The price of radium rose from £2 to £5 per milligramme in 1904, to £12 in 1906, £27 in 1910, £30 in 1912, and £36 in 1914; by 1922 it had dropped to £22 per milligramme, with a further fall to £14 in 1923, the price in 1935 being £12, and the market price in 1937 was approximately £6 per milligramme, being forced down by the Canadian supplies. In 1938 the Government was reported to have placed an order in Canada for 11 g. of radium at a cost of £200,000.

A large supply of radium for hospital use was purchased by the Radium Commission set up in 1930. This radium is loaned to various centres under controlled conditions. The total amount put at the disposal of the Radium Commission by the National Radium Trust was approximately 21 g. which is on loan to thirteen national centres, and five regional centres, the M.R.C. and the N.P.L. The Commission also exercises jointly with the King's Fund control over 3 one-gramme units at the Cancer, Middlesex and University College Hospitals. The total quantity of radium available in the country in September 1939 was over 100 g.

Radium Standard (International). This consisted of 21.99 mg. of radium chloride made by Madame Curie and stored in a thin sealed glass tube at the Bureau des Poids et Mesures at Sèvres, Paris. Another International Standard was kept at the Academy of Sciences at Vienna. Duplicate standards were in the hands of governments of other countries.

The **British Radium Standard** is kept at the National Physical Laboratory, Teddington. It contains about 20 mg. of pure radium chloride. The activity of specimens should be expressed in terms of metallic radium instead of bromide, to prevent misunderstanding regarding the presence of other elements as well as the $2\text{H}_2\text{O}$ in crystallised radium bromide. The activity is proportional to the number of radium atoms present and is independent of the chemical compounds in which they are combined.

Standard Solution of Radium. Sealed tubes are made containing 1/100,000 mg. of radium as metal in 10 ml.

In estimating radium in samples of its salts use is sometimes made of a measurement of emanation, if the amount is too small for direct estimation by γ ray methods. The specimen should be dissolved in 100 ml. of water, some pure hydrochloric acid being added; of this 5 ml. should be diluted to 1 litre, making 1 mg. in 20,000 ml. of water, or 1/20,000th of a milligram in 1 ml. If 1 ml. be diluted to 50 ml. the strength will be approximately that required for the electroscope, and a comparison may be made with the standard. To calculate the radium to pure crystallised bromide multiply by the factor 1.867.

Characters of Radium. Radium should be placed below barium in the Mendeléeff series, and on the same line as thorium and uranium. These three radioactive elements have the highest atomic weights. Radium is divalent. Its spectrum resembles those of the alkaline earths. Its emanations affect a photographic plate and cause fluorescence in substances such as barium platinocyanide, zinc sulphide, calcium sulphide, fluorspar, willemite, diamond and ruby. They promote certain chemical reactions, such as converting oxygen into ozone, and discolour paper and glass. They also affect the skin and produce painful, slowly healing sores.

Electrical Properties of Radium. The rays emitted by a highly active preparation discharge a charged gold-leaf electroscope even through an inch or more of iron, zinc or lead—5 milligrams will do this at a distance of a few yards. This occurs whether the charge on the leaves be + or -. All three types of radiation from radium have the effect of ionising air in the electroscope, breaking the molecules into constituent ions, each of which is electrically charged + or -. These charged particles collide with the charged gold leaves, and such as are of opposite sign to the charge on the leaves neutralise a corresponding amount of electricity on the leaves.

The rays are of (at least) three main types:—The α -particle, which is a positively charged atom of helium, is the least penetrating of the three, and is absorbed by thin screens of paper or mica, or by about 3 inches of air. It is expelled with a velocity equal to about one-twentieth that of light. The β -particle is an electron or unit of negative electricity with an apparent mass of about $\frac{1}{1847}$ of the hydrogen atom. Its velocity of expulsion varies from one-third to almost equal to that of light, and it can penetrate a sheet of lead of about 3 mm. thickness. The gamma-ray carries no charge and, like X-rays, is a wave motion propagated with the velocity of light and is much more penetrating than either the α - or β -particles.

The average life of radium is probably about 2000 years. In other words $\frac{1}{2000}$ part of a given mass of radium changes annually.

The genetic relation between uranium and radium has been established. There is always a definite proportion of radium to uranium present in uranium minerals—for every 1 part of radium there always exist 3,000,000 parts of uranium. $1/\lambda$ for uranium is 8,000,000,000 years (current figure gives 6.75×10^9 , i.e. 6,750,000,000 years). The average life is always 1.443 times the time T known as the *period* required for the quantity of the element to be diminished to $\frac{1}{2}$ value. Thus the $\frac{1}{2}$ value of radium is 1580 years and $1580 \times 1.443 = 2280$ years, i.e., the average life of radium. For the emanation the average life = $3.85 \text{ days} \times 1.443 = 5.52 \text{ days}$.

Action of Radium. All the rays emitted by radium can produce effects on living tissue. These effects are similar in nature whichever type of radiation (alpha, beta or gamma) is used, and all degrees of cell damage may be produced up to the complete

death of the cell. Although it is true that there is no tissue in the body that cannot be destroyed by exposure to any of the radiations of radium in sufficient dosage, the sensitivity of the different tissues and organs varies greatly. Among the more sensitive of these are the essential glandular cells of the testes and ovaries, the leucocytes, especially the lymphocytes and lymphatic tissues generally, the hæmopoietic tissues, the skin and mucous membrane, the secreting cells of the salivary glands, the conjunctiva and cartilage.

Among the tissues more resistant to the radiations of radium are muscle, nerve tissue, the liver and the kidneys. On the skin an inflammatory reaction is caused which, when it stops short of vesication, is termed "erythema." The erythema is accompanied by epilation of hairy parts and depression in the activity of sweat and sebaceous glands. It subsides in a few days, and an almost normal skin remains, perhaps somewhat abnormally dry. Larger doses cause vesication, and still larger doses necrosis of all the layers. Ulcers so produced are extremely difficult to heal and are often very painful. The effects described are those produced by large doses applied in a short period of time, such as are used in radium therapy. Repeated small doses, such as are sustained by those who have to handle radium in the course of their work, produce somewhat different effects, namely, dryness, hyperkeratosis and warts, fissures, and in some cases, eventually, epithelioma. In both types of exposure, but more commonly in the chronic type, telangiectases may follow.

The effects upon mucous membranes are principally inflammatory. Cystitis, proctitis, vaginitis and other inflammations of the mucous membrane are commonly seen after therapeutic irradiation by radium, and perforation of hollow organs may follow. The action upon the sexual organs results in the destruction of the spermatogenic and oögenic cells, with consequent sterility. So far as is known, the internal secretory cells are not affected, and, in the male, sterility is not accompanied by impotence. Appreciable changes in the blood cells are only observed after large doses or repeated exposure to small doses as in the case of persons who habitually have to handle radium. The first effects are upon the leucocytes, the total number of which is reduced. While all forms of leucocytes suffer some reduction in their number, it is found that the lymphocytes suffer most, and these cells may form only 5 to 10% of the total count. The red cells and hæmoglobin may be moderately reduced, the colour index tending to fall. However, in chronic exposure to radium radiations there is probably a stage in which both red cells and hæmoglobin are increased slightly above normal, and the colour index may be above unity. Aplastic anæmia is said to be an end-result of repeated exposure to small doses of radium radiations.

(For further details of the action of radium, including its action on pathological tissues and the mode of application, see under X-ray and Radium Therapy, p. 1047.)

Uses. Radium is widely used in therapy, in the production of luminous paints, and occasionally for radiographic detection of flaws in metal castings. The war-time demand for aircraft has greatly increased the demand for luminous paint for instrument dials and it seems fairly certain that the consumption for this purpose now greatly exceeds that for medical use.

In luminous paints composed of radium and zinc sulphide, the zinc sulphide undergoes rapid deterioration—the rate of decay in luminosity is proportional to the amount of radium present, but not exactly proportional. Radium paint containing 0.4 mg. of radium bromide or its equivalent (in 1 g. of zinc sulphide) has a luminosity of about 0.03 foot candles, while a paint containing half this amount of radium has more than half the luminosity.

Dosage. For surface application, the dosage depends upon a great many technical factors, such as the distribution of radium in the applicator, the wall-thickness of the applicator and the distance of the applicator from the surface. The dosage is largely a matter for experimental determination with each applicator. The erythema dose is a valuable guide in this connection and, generally speaking, full erythema doses are required in the treatment of malignant conditions. A common method of expressing dosage is in "milligram-hours," that is, the product of the number of mg. of radium used and the number of hours for which it is applied. This statement of dosage is of little value unless it is accompanied by all the other details of the application. The same applies to the intracavitary and interstitial methods. As a rough guide, with a total quantity of 50 mg. of radium, filtered by 0.6 mm. of platinum and evenly distributed in needles throughout the cervix uteri and parametria, an average total dose would be from 5000 to 6000 milligram-hours.

Artificial Radioactivity. Very recently it has been discovered that many substances may be made radioactive artificially by bombardment with neutrons, i.e., particles having high speeds and a mass nearly that of a proton but no net electrical charge. Such neutrons may be produced by α rays or γ rays on beryllium. This induced radioactivity is in general very feeble but is a real **artificial production of radioactive materials** and must not be confused with the "induced activity" above. We now have definite evidence of the production of artificial radioactivity.

Early in 1934 M. and Mme. Curie-Joliot demonstrated the possibility of manufacturing radioactive substances. They found that when boron or other substances were bombarded with the swift alpha particles, or nuclei, of helium atoms, emitted from the naturally radioactive substance polonium, they emitted positive electrons, and the emission of these electrons continued after the polonium source of bombarding particles had been removed. The radioactive atoms produced in the boron consist of a form of nitrogen which they named "**radio-nitrogen**." Cockcroft and Walton later demonstrated that radio-nitrogen could be manufactured in their chemical apparatus by bombarding carbon with protons, or nuclei, of hydrogen atoms. It is important to realise that this artificial radioactivity is rather different from the natural radioactivity; radio-nitrogen emits positive electrons when it disintegrates—natural radioactive substances emit wave radiations, the nuclei of helium atoms, and negative electrons.—*Brit. med. J.*, ii/1934, 689.

Lawrence reports two years' experience of treatment of a case of chronic leukaemia in which the effects of a single dose of "**radio-phosphorus**" have been similar to those of a course of X-ray therapy. The maximum dose of sodium phosphate was 3 g. containing up to 8 millicuries of radio-phosphorus, which would be equivalent to about 3r daily whole-body irradiation for a fortnight. Lawrence has also treated two cases of polycythæmia vera with good

results. The dosage used so far has been small, but from X-ray experience it would appear that 100 mc. or more of radio-phosphorus could be tolerated which would give a sufficiently localised intensity of radiation in bone to destroy multiple myelomata or metastatic carcinomatous deposits.—*Brit. med. J.*, i/1941, 368.

Clinical studies with radioactive phosphorus.—*Brit. med. J.*, ii/1941, 733.

X-RAY DIAGNOSIS

X-rays, or Röntgen rays, were discovered by Röntgen in 1895. They are similar in nature to light, heat and wireless waves but of considerably shorter wave-length, those used in X-ray diagnosis having a wave-length between 0.6 and 0.124 Ångstrom units.

Method of Production. They are produced in medical practice in an X-ray tube. A modern X-ray tube is a vacuum tube and contains a filament which is heated red hot. Opposite the filament is the target, which consists of a piece of tungsten mounted on a block of copper.

The filament is made negative, relative to the target, by the application of a suitable high voltage, with the result that the negatively charged electrons travel from the filament to the target. Here they are stopped abruptly, with the consequent generation of much heat (which is dissipated by a cooling system) and also of X-rays.

The region where the X-rays could escape through the glass of the tube is usually surrounded by lead in which a small window is left, so that the emergent beam is confined to a definite direction (so called "self-protected" tubes).

The electrons from the filament are focussed on the target, so that only a small area on this is used for the production of X-rays. Tubes are often rated according to the size of this focal spot, a fine focus tube having an effective area of less than 2.2 sq. mm., medium focus tubes being about 3.1 sq. mm. and broad focus 4.1 sq. mm. or more. They are also rated according to the number of watts (milliampere \times kilovolts R.M.S.) which can be applied to the target for one second. The above-mentioned focal spots would correspond approximately to 2.5 kw., 6 kw. and 10 kw. ratings, with fixed target tubes.

Sometimes the targets are made to rotate and then the electron stream strikes a different part of the tungsten target each moment. These tubes have a much higher kw. rating for a given sized focal spot. Tubes may now be obtained in earthed metal casings and with suitable shock-proof leads, so that danger from electric shock should no longer be present.

Accessory Apparatus. A suitably regulated transformer is required to raise the voltage of the A.C. mains from 100 to 480 volts, according to supply available, to between 40,000 and 100,000 volts, the figure being regulated according to the part being radiographed.

Other controlling devices required are accurate timing devices with control ranging from 1/100 second to 10 seconds in powerful sets, and 1/10 second to 10 seconds in smaller sets. Arrangements are made for the suitable control of the filament current, which in turn controls the current through the tube.

A large set will take from 12 to 30 kw. for one second from the main supply and usually requires a special mains cable. Small mobile units can be worked off ordinary power plugs and will take about 3 kilowatts or 15 amperes on a 200 volt A.C. supply. Three-pin plugs, with one pin for earthing, should always be used.

Portable X-ray Units. Small portable sets are available to work off electric light supplies, but are, of course, very low powered. They are of use for fracture work in the patient's home and some chest work, but if a more powerful set is required, complete units are available in London generating their own electricity. Amongst others may be mentioned the Order of St. John and the British Red Cross Mobile X-ray Department, 12 Grosvenor Crescent. Films taken by them may also be sent to the British Institute of Radiology for interpretation by senior radiologists.

Cineradiography. A method of examining the movements of joints and organs of the living body by taking a photograph of the screen image with a cinematograph camera.

To obtain a sufficiently brilliant screen image to record on a cinematograph film in the fraction of a second one must employ an X-ray plant giving sufficient output, and an X-ray tube capable of withstanding the heavy currents that are necessary for the production of a powerful beam; the camera lens must have as large an aperture as possible to allow the maximum of light to pass through it; the film employed must be sensitive to the particular wave-length of light emitted from the fluorescent screen used; it is necessary to cut off the direct beam of X-rays passing through the screen, so that only ordinary light can reach the film; protection must be provided for the patient so that he will not suffer from undue exposure.

The advantages of cineradiography are: it enables one to obtain a rapid, inexpensive and permanent record of the functioning of active organs; the continuous "band" enables one to study movement for an indefinite period; the permanent records of movements may be used for diagnostic purposes, comparison with former records, teaching purposes, transmission abroad or elsewhere for obtaining specialists' opinion, or for information as to the condition of the patient in the past. (A description of the apparatus and the technique involved).—R. J. Reynolds, *Brit. J. Radiol., N.S.*, 1934, 415; *Brit. J. Radiol. (Röntg. Soc. Sect.)*, 1927, 33.

Mass Radiography. For examination of very large numbers of chests, such as in recruits or factories, the fluorescent screen image of each patient is photographed on one frame of a cine-film and named or numbered. Later the film is developed and the photographic images projected on a screen for interpretation. Provided large numbers are being dealt with, the cost is much less than using full-sized X-ray films. Described by S. F. Dudley and W. Fitzpatrick, *Proc. R. Soc. Med.*, 34, 401, and J. V. Sparks, *Brit. med. J.*, 1941, 4198.

Properties of X-rays.

(a) **Penetration.** X-rays have the power to penetrate matter to a degree which is dependent upon the quality or wave-length of the ray and the density of the material. The shorter the wave-length of the ray the greater the degree to which a given material is penetrated by it; and the denser the material the less the degree

to which it will be penetrated by a given wave-length. Hence the possibility of X-ray photography, the rays being transmitted by the different structures of the body in different degrees dependent upon their several densities.

(b) *Absorption.* The fraction of the radiation which is not transmitted through the object irradiated is said to be absorbed. Such absorbed radiation is capable of producing:

(c) *Biological effects* in the animal body. (See section on X-ray treatment.) Certain chemical changes may also be induced in suitable media, among the most important of which are:

(d) *Photographic effects* and

(e) *Fluorescence.* The best known substance in which fluorescence is produced by X-rays is barium platinocyanide which was employed in the manufacture of fluorescent screens.

Fluorescent Screens. Certain substances give off visible light when exposed to an X-ray beam and hence, where differential absorption occurs, an image can be seen on such a screen, e.g., radio-opaque barium in the alimentary tract. Zinc sulphide or calcium tungstate are used, spread in a thin layer on cardboard. This should then be covered with a lead glass which will let the visible fluorescent light through but absorb the X-rays. The glass should absorb X-rays to the same extent as 2 mm. of lead would do.

"**Fluorazure,**" an intensifying screen, allowing more rapid X-ray exposures, is made from specially refined zinc sulphide, and gives an intense azure blue fluorescence. Cadmium tungstate screens made it possible to reduce the ordinary exposure time to about 1/10th, but with fluorazure this could be reduced again to about 1/3rd or 1/5th the exposure with the tungstate screen.—N. S. Finzi, *Brit. med. J.*, ii/1932, 980.

Protection in Radiography. *Recommendations of the X-ray and Radium Protection Committee.* 1. No person should be employed as an X-ray or radium worker whose blood (as tested by a complete blood count) or general health is unsatisfactory. 2. Before beginning work or training the normal leucocyte level should be found. If none of the total counts reaches 6000 per c.mm. and none of lymphocyte counts reaches 1200 per c.mm. the worker should not be accepted. 3. Periodical total and differential blood counts should be made during the morning period every 6 months in X-ray workers and every 3 months in radium workers. 4. If there is a decided and sustained drop in the total leucocyte or lymphocyte count the worker should cease work and be placed under treatment, and every care taken on resumption of work to prevent a recurrence.—*Brit. med. J.*, ii/1933, 838.

Systematic blood counts have been carried out at intervals of two to four weeks on 32 workers in radium and X-rays. The periods of observation vary from six months to three years according to the duration of each individual's appointment. It has been found that there is only one sign of early over-exposure, which is common to all workers. This is a leucopenia due to a reduction in the number of circulating neutrophils. There is

evidence that different individuals vary in their susceptibility to the effects of irradiation, and those who are more sensitive exhibit an absolute lymphocytosis with an absolute neutropenia as a first sign of over-exposure. Others, who are less sensitive, exhibit a lymphocytopenia with a monocytosis under similar circumstances. Eosinophilia has frequently been seen as a result of over-exposure, and abnormal or embryonic leucocytes have occasionally been seen in the blood of certain workers. Holidays of less than four weeks do not appear to be of value in restoring the leucocyte count of an over-exposed worker to a normal level.—D. R. Goodfellow, *Brit. J. Radiol. (B.A.R.P.Sect.)*, 1935, 752.

Recommendations by the X-ray and Radium Protection Committee for electrical precautions in X-ray rooms.—*Brit. med. J.*, i/1934, 294.

Use of X-rays in the manipulation of fractures. Memorandum drawn up by the British X-ray and Radium Protection Committee.—*Brit. med. J.*, i/1942, 84.

All persons working with X-rays should consult the above recommendations, copies of which can be obtained from the British Institute of Radiology, 32 Welbeck Street, London, W.1. It should be remembered that although tubes are described as "self protected," this is only a relative term, and the operator should avoid exposing himself to any part of the direct beam. It must also be remembered that there is considerable scattering of the primary beam from the patient, and it may be necessary to protect the operator from such scattered rays.

The operator must have sufficient knowledge of his apparatus and X-ray dosage to avoid giving a dangerous or excessive dose of rays to the patient, whether while screening or by an excessive number of exposures. He should also beware of conducting a prolonged X-ray examination on any patient who has been previously X-rayed within 14 days or has had, within some months, heavy dosage X-ray treatment to the part being radiographed.

Using self-protected tubes with $\frac{1}{2}$ mm. filter of aluminium, and working at a tube-patient distance of 24 inches and with a kilovoltage of about 80 k.v.p., 1500 milliampere seconds is a safe dose. This would allow 5 minutes of screening at 5 ma. At 12 inches one could screen safely for $1\frac{1}{2}$ minutes, the dosage of X-rays varying as the square of the distance.

For diagnosis, using less than 110 k.v.p., protective materials should be of 2 mm. lead or equivalent to this, other materials in use being lead glass for the front of the fluorescent screen or for the operator's window. Barium plaster may be used for walls.

About 20 mm. ($\frac{3}{4}$ inch) of steel plate found to give protection equal to 3 mm. of lead. A barium sulphate mixture required 60 mm. to equal 3 mm. of lead. P. J. NEATE's formula, one-third coarse barium sulphate, one-third fine, one-third cement, gave somewhat better results. The open lead-glass bowl affords no protection in many directions. Sheet-glass, of lead value 0.12 per mm., well spoken of—obtainable as thick as 18 mm.—G. W. C. Kaye and E. A. Owen, *J. Röntgen Soc.*, 1923, 169.

An exhaustive study of protective materials at the Nat. Phys. Lab. for the Protection Committee. Numerically, 1 mm. of the following is equivalent to the stated thickness of sheet-lead in mm.

| | | | |
|---------------------|--------------|------------------|---------------|
| Lead glass .. | 0.12 to 0.2 | Woods .. | 0.001 or less |
| " rubber .. | 0.25 to 0.45 | Baryta plaster.. | 0.05 to 0.13 |
| Bricks and concrete | about 0.01 | Steel .. | 0.15 |

These are relative to tungsten X-rays generated by 100,000 volts.—G. W. C. Kaye, *Phys. Soc. and Röntgen Soc. Joint Publicn.*, Feb. 23, 1923.

Protection from scattered rays need not be quite so heavy; (1 mm. lead equivalent being recommended) which will apply to lead rubber aprons, etc. Lead rubber gloves must, unfortunately, be pliable and so are also of less protective value.

Protective materials and equipment can be tested by the National Physical Laboratory on request.

Nitrous acid is certainly not present in the air of the X-ray room in sufficient amount to produce ill effect upon the patient or operator. However, in favourable conditions ozone may be produced in amounts as much as seven times as large as Konrich's figure for the minimum quantity (0.5 mg. per millilitre of air) which produces exhaustion, blood changes, etc.

Röntgen "gas" poisoning resembles ozone poisoning.—*J. Röntgen Soc.*, 1921, 155.

Photographic Aspects. Most modern films are duplitised, i.e., coated with emulsion on both sides. They are now made with the usual clear base, or with a blue base, giving a bluer tint to the unexposed parts in a developed film.

Films are also made with a matt base, which is of use to those without adequate viewing boxes, but the fine detail is not quite so clear as with the other two.

In order to be able to give rapid exposures, films are packed and exposed between intensifying screens which, by glowing like a fluorescent screen, enhance the direct effect of the X-rays on the film by a light effect. The only disadvantage is a slight loss of fine detail and hence, for limb work, plain films are often preferred.

Intensifying screens are made of cadmium or calcium tungstate or zinc sulphide.

Films are supplied in light-proof covers (double-wrapped) or in boxes (single-wrapped). The latter are taken out of their box in the dark room and placed between the intensifying screens, which are kept in a special holder called a cassette, which is light-proof when closed.

Paper. Instead of using the sensitive emulsion on a celluloid base, it can also be obtained on a thick paper. These paper films are less expensive than ordinary films, and are of use for certain types of work where very fine detail is not required, e.g., barium meals and repeat fractures.

They may be used with or without intensifying screens, and are developed in the same way as X-ray films. When used with intensifying screens, only one screen is necessary since the emulsion is only on one side of the paper.

Developer for X-ray Films. Hydroquinone 320 gr., potassium bromide 200 gr., metol 80 gr., sodium sulphite (crystals) 16 oz., sodium carbonate (crystals) 8 oz., water up to 80 fl. oz.

Films should be developed for 5 minutes at 65°F. The solution

will keep for some months. A 3-gallon tank will develop 200 films, taking a mixed batch of all sizes.

Fixing Bath for X-ray Films. Sodium hyposulphite 1 lb., sodium or potassium metabisulphite 1 oz., water 40 fl. oz.

A film should be fixed in a few minutes and shows no yellow emulsion on it when fixed. Films should be washed in running water for half an hour after fixing.

Contrast Media. A detailed radiographic image can only be obtained where differences of radio-opacity occur.

Cartilage is so similar in radio-opacity to skin and muscle, that normally it cannot be seen as a separate shadow. In order to make the hollow viscera visible, contrast media are introduced into them and hence they can often be outlined.

The following are some of the contrast media employed:—

Alimentary Tract. Barium sulphate.

For the œsophagus. A thick paste made of pure barium sulphate and water is very satisfactory. A tablespoonful of the paste is placed in the patient's mouth, and he is then requested to swallow it, the operator watching its passage on the fluorescent screen. If the patient complains of great difficulty in swallowing it is safer to use the barium emulsion as for the stomach, as the thick paste may stick and cause complete obstruction for a time if there is much œsophageal narrowing.

Stomach and Duodenum. Pulvis Barii Sulphatis Compositus (B.P.C.).

A good alternative formula for barium meal work is:—Barium sulphate 10 oz., vanillin 2 gr., saccharin 2 gr., tragacanth 60 gr., distilled water to 20 fl. oz.

The gum and barium should be mixed as a powder, and the water added gradually.

The usual technique is for the patient to swallow a mouthful of the mixture and the mucosal pattern of the stomach is then examined. Later the stomach is filled out with barium, and an adult may require 15 to 30 oz. of the emulsion. No food or drink should be taken for 6 hours before a stomach examination.

For children, some prefer bismuth oxychloride, but thin barium emulsion diluted half strength with water is quite suitable. Only a small quantity is given and the only danger is stagnation in the colon. If followed by a purgative after a suitable interval, this danger can be avoided.

Small and Large Intestine. The above emulsions are suitable.

Barium Enema. Barium sulphate 6 oz., tragacanth 40 gr., water to 20 fl. oz.

Or the barium meal emulsions may be used diluted with water.

The stomach can be outlined to some extent by giving a seidlitz powder and outlining it by carbon dioxide (low density contrast media) and the colon by air inflation.

Appendix. The bowels must previously be thoroughly evacuated by a suitable purgative. It is recommended to administer the barium meal mixed with two teaspoonfuls of epsom salts. The appendix is seen after from 6 to 24 hours, the best time being after from 10 to 12 hours. The next examination follows 24 hours after ingestion of the meal, and further examinations, if necessary, at 24-hour intervals, the colon also being examined at the same time.—A. Orley, *Brit. J. Radiol.*, 1935, 487.

Benzedrine sulphate has been found of great value in facilitating the roentgen study of the gastro-intestinal tract by diminishing or abolishing spasm. After its administration the relaxation of the sphincter permits the passage of a broad stream of the barium sulphate mixture through the pylorus and duodenum. Gastric tonus is slightly lessened but peristaltic action is not interrupted. The dosage for the average patient is 30 mg. orally taken with a few sips of water after the opaque meal. The effect on spasm is almost immediate and is unattended by any side effects of importance. The effect reaches its height in from 15 to 30 minutes, lessens within an hour and gradually disappears.—A. Myerson and M. Ritvo, *J. Amer. med. Ass.*, ii/1936, 24.

Urinary Tract. For retrograde pyelography, radio-opaque catheters should be used, and a sterile 20% solution of sodium bromide or 15% sodium iodide can be injected up the catheters to outline the renal pelvis or ureters.

The normal renal pelvis holds 5 to 15 ml., while a large hydro-nephrosis may require 100 ml. to outline it. If large quantities are used, they should be drained out before the catheter is withdrawn. The bladder may be outlined with 10% sodium bromide. Occasionally, negative contrast media (air or oxygen) are used to demonstrate the renal pelvis.

For taking pyelograms at the Gt. Ormond St. Hospital for Sick Children a 13% solution of sodium iodide is used but it has some irritating effect on the kidneys and in practically every case there is blood passed in the first urine after pyelography. The blood is usually small in amount and does not persist for more than a few hours; a few hyaline and granular casts also occur. Iodoxyl, which is not irritating to the kidneys, may be used but the expense is very great.

For cystography 5% sodium iodide is quite strong enough but when back-pressure kidneys are present it may produce severe iodism or even uremia. There is no objection to making bilateral pyelograms if required—in fact one usually does so to avoid giving a second anaesthetic.

As the child is unconscious great care must be exercised not to over-extend the pelvis and a satisfactory method to ensure that the correct amount is given is to inject a fixed amount according to the size of the child. The normal pelvis holds a little under 1 ml. for each year of life, with a maximum of 5 or 6 ml. in older children. The dose corresponding to the age of the child is given and a photograph taken and developed immediately. Some indication of the size of the pelvis is obtained and, if necessary, a second injection is made of an amount estimated to be enough to give a satisfactory picture. A second X-ray photograph is then taken.—O. L. Addison, *Proc. R. Soc. Med.*, 1936, 29, 1296.

Thorotrast, a stabilised thorium dioxide sol containing 25% of thorium dioxide, diluted with water or physiological salt solution, is used in retrograde pyelography in a dilution of 1 in 2; in cystography, 1 in 5; and in examination of tracts of fistulae, 1 in 1.—*Brit. med. J.*, ii/1932, 112.

Intravenous Pyelography. Iodoxyl or diodone, when injected intravenously, are secreted rapidly by the kidneys and give good shadows of the renal pelvis, calyces, ureters and bladder.

Adult dose: 20 ml. iodoxyl is non-toxic and non-irritant to the tissues. Should not be used in uræmic patients.

The patient should restrict fluid intake for several hours before, and no diuretics, such as tea or coffee, should be taken for 12 hours. First skiagram taken 2 to 5 minutes after injection.

Sixty per cent. will be excreted in the first half hour, if the kidneys are functioning normally.

Intravenous pyelography has proved a great advantage in children in the investigation of urinary disorders, for it is easily carried out, avoids an anæsthetic and often a difficult or impossible instrumentation. Iodoxyl may be given in doses from 8 ml. in infants to 12 ml. or more in older children. It has little or no toxic effect. It is very useful in showing the presence of double ureters. —O. L. Addison, *Proc. R. Soc. Med.*, 1936, 29, 1297.

For further references to iodoxyl and diodone, see Vol. I, p. 652.

Gall-Bladder. Sodium tetraiodophenolphthalein (Iodophthalein B.P.), when in the blood stream, is secreted in the bile by the liver. If it reaches a normally functioning gall-bladder, it is stored and concentrated, and outlines the gall-bladder on a subsequent radiogram.

Intravenous injection. Dose: 3.5 g. in 10% solution in sterile water (i.e., 35 ml.).

It is very toxic locally and care must be taken that none escapes into the tissues during injection.

The patient should have no food or drink after supper in the evening. The injection is given early next morning and films are exposed 4 and 8 hours later.

Oral method. This is now almost as accurate as the intravenous method, 98% of normal gall-bladders producing a good shadow, and it is, therefore, usually the method of choice.

Dose: 3.5 to 5 g. dissolved in an acid medium.

The patient takes a fat-free supper at 7 in the evening and the dye is best taken directly after in half a wine glass of grape or orange juice. No further food or drink should be allowed until after the films are taken, about 15 and 17 hours later. Sometimes a final film is taken an hour after a fatty meal to see how the gall-bladder is emptying, or to detect small stones in it.

Contra-indications. Severe liver disease or marked obstructive jaundice. In cases of gastric or duodenal ulcer, non-filling is not a conclusive indication of a lesion in the biliary tract, if the gall-bladder fails to fill with and concentrate the dye.

For further information concerning the diagnostic use of iodo-phthalein, see Vol. I, p. 816.

Genital Tract. Iodised oil may be used, by injection into the uterus, to outline the uterus and fallopian tubes. Pregnancy is a contra-indication.

It may also be injected into the seminal vesicles, but the method is not much used in this country.

Peritoneum. Oxygen may be injected into the peritoneal cavity, which is then radiographed. This procedure is mainly used for localisation of abdominal tumours, where other methods have failed to give the desired information.

Spleen and Liver. Thorotrast, when injected intravenously, is absorbed by the liver and spleen, and these become denser radiographically. It may demonstrate large secondary deposits in the liver, as it is not absorbed by these.

Dose: 10 ml. first day; 15 ml. second day; 20 ml. third day and then to a total of 50 to 75 ml.

Although death has rarely occurred as a result of Thorotrast, it is reasonable to suppose that even such a feebly radioactive substance as thorium must have some effect. It has been shown that the liver and spleen are still easily visible radiographically 2½ years after administration of Thorotrast.—W. P. A. Murphy, *Brit. med. J.*, ii/1933, 249. Use unjustifiable except in malignant disease with hopeless prognosis.—J. F. Brailsford, *ibid.*

Dangers of faulty diagnosis due to retention of remnants of thorium, traces being found in the kidneys up to 2 years after pycelography.—K. Scheele, *per Brit. med. J. Epit.*, i/1934, 30. The delayed effects of Thorotrast: a warning against its employment.—*ibid.* 40.

In the present immature state of our knowledge of the biological effects of radiations it is unwise to introduce into the body radio-active material the rate of degeneration and the natural elimination of which are known to be slow, unless removal from the tissues is under the control of the practitioner.—*Rep. med. Offr Minist. Hlth, Lond.*, 1935, 116.

Avoidance of the use of this substance may be no less a means of prevention of cancer than are others of a more positive nature.—*Rep. med. Offr Minist. Hlth, Lond.*, 1936, 119.

Mastitis following use of a thorium dioxide injected for diagnostic purposes. Report of a case.—B. F. Sowers and J. C. Masson, *Proc. Mayo Clin.*, 1937, 529.

Trachea and Bronchi. Iodised oil can be introduced by several methods:—Puncture through crico-thyroid membrane, laryngeal catheter via nose, or simply dropped on back of tongue after anaesthetising with cocaine spray; also down bronchoscope during bronchoscopy.

For a full description of these various diagnostic procedures using Iodised Oil, see Vol. II (21st Edn.), p. 192; see also Vol. I, p. 650.

Dose: 10 to 20 ml. depending on the regions which it is required to fill at a sitting.

Pleural Cavity. Artificial pneumothorax is sometimes used for diagnosis of suspected innocent tumours (fibroma of chest wall).

After empyema, iodised oil may be injected via the sinus to see that the cavity is closing up well.

Iodised oil is also used to outline the parotid duct and its branches and the submaxillary and lachrymal ducts.

Nasal Accessory Sinuses. Iodised oil, viscous or non-viscous.

Spinal Theca. 1 to 2 ml. of iodised oil injected by passing needle into cisterna magna via space between atlas and occiput.

Examination conducted with the patient sitting up. May also be injected by ordinary lumbar puncture. A tilting X-ray couch is necessary to manipulate the oil up and down.

Skull. Air may be injected by lumbar puncture or directly into the cerebral ventricles via a small trephine hole, and the cerebral ventricles can thus be outlined on a radiogram.

Colloidal thorium dioxide is of great value as a contrast medium for ventriculography. It is freely miscible with the ventricular fluid, is of high specific gravity, finding its way into the recesses of the ventricular system, and on account

of its high radiopacity needs to be used in relatively small amounts. It is eliminated, in normal cases, within four hours and is so inert that it provokes only a mild inflammatory reaction. Most important of all, it preserves the supporting fluid cushion of the brain and avoids the serious constitutional effects of air ventriculography.—W. Freeman, H. H. Schoenfeld, and C. Moore, *J. Amer. med. Ass.*, 1/1936, 99.

Arteriography. Thorotrast, 10 to 20 ml., to outline arteries to limbs or brain. Rapid skiagram taken during injection. Veins may also be outlined by this means.

Sinuses. May be outlined with iodised oil, or suspensions of bismuth oxychloride. Iodised oil tends to drain away better.

Joints. These are sometimes outlined by oxygen or iodoxyl injected into the synovial space.

X-rays are also used without the help of contrast media for the examination of the bones, muscles and viscera, etc., and for the localisation of radio-opaque foreign bodies. It should be noted that glass may contain some lead and is then opaque, or may be of a density so near to the skin and muscle that it cannot be demonstrated by X-rays.

The teeth are best examined by special small dental films, which are placed in the mouth and held against the teeth either by the patient's own forefinger or by special dental holders.

A skiagram gives only a two-dimensional image, and where possible, a view at right angles should be obtained to give a composite three-dimensional view of the part. Where this is not possible, stereoscopic films can be taken. Two films are taken, the tube being moved 1/10 of the tube-film distance between each film. The films are then viewed in a mirror stereoscope, or with stereoscopic binoculars. The method entails judgment on the part of the observer and is not as good as two films at right angles, where these can give the required information.

Legal ownership of X-ray films. It is almost certainly the custom that the films remain the property of the medical man who takes them or under whose direction they are taken. Although many radiologists recognise an informal right in the patient to have the films lent to a practitioner, who may at a future time have charge of his case, for his guidance in its treatment, it is unlikely that the patient could enforce such a right at law.—*Brit. med. J.*, 1/1934, 83.

There are very few radiologists who do not send either a film or a print to the patient's doctor and a very large proportion of the patients are subsequently presented with the films. It is probable that a court of law would find there was no universally accepted custom or that it was usual for the patient to be presented with his films.—C. H. C. Dalton, *ibid.*, 172.

X-RAY AND RADIUM THERAPY

Radiations have proved valuable mainly in two sets of conditions : (1) In acute and chronic inflammatory conditions; (2) In the treatment of new growths, especially the malignant variety.

The mode of action of radiations in causing the resolution of inflammatory conditions is not clear. In doses applicable to the human body they have no effect on bacteria. It has been suggested that in acute inflammatory conditions the rays, by

affecting a much wider area than is involved in the inflammation, stimulate the production of non-specific antibodies on a larger scale than would be the case with the limited inflammatory lesion alone. In chronic inflammatory conditions, the mode of action may be by causing the dissolution of the accumulations of the small round cells which characterise these conditions, thus allowing a more active hyperæmia, i.e., a mild acute inflammatory reaction. The repeated application of the radiations at intervals of several days, by causing these repeated mild inflammatory reactions, tends to overcome the infecting agent.

Considerable difference of opinion exists as to the mode of action of radiations in malignant disease. By some authorities the effect is regarded mainly as an indirect one, but there is now little doubt that in the majority of cases the direct damage to the cancer cell is of greater importance, at any rate in so far as the immediate result is concerned. The value of radiations in the treatment of cancer depends upon the greater sensitivity of the cancer cells in relation to the normal body cells. This may be due to the much greater activity of the cancer cells compared with the normal cells. Such a view is supported by the fact that the normal body cells themselves exhibit a degree of sensitivity which is roughly proportional to their activity. Thus, the essential testicular and ovarian cells and those of the skin, which are constantly renewing themselves, are among the most sensitive cells in the body.

Constitutional Effects of X-ray Treatment. The term "X-ray sickness" is applied to the "symptom complex" which may follow the application of X-rays in moderate or large doses. It occurs most commonly after the irradiation of the upper abdomen, but it may also occur after the irradiation of any other region if the volume of tissue exposed has been sufficiently great. In its lesser degrees, the condition is characterised by a feeling of malaise, headache and nausea. Severe degrees are accompanied by vomiting, which may be severe and even intractable. In the worst cases, continued vomiting may lead to the exhaustion and death of the patient. The causation of the condition is obscure, but it is probably in the nature of a protein poisoning due to the liberation of the products of cell-destruction. The condition in its milder forms is treated by the exhibition of gastric sedatives such as bismuth and hydrocyanic acid, or tincture of iodine. In the more severe degrees, chlorbutol or even morphine may have to be given. In the worst cases, benefit has been claimed from the infusion of normal saline into the veins.

The administration of large doses of vitamin C has been recommended during irradiation by Carrié (*Strahlentherapie*, 1938, 183). This author states that the leucopenia due to X-rays can be favourably influenced by the administration of vitamin C, and he also recommends that this vitamin should be taken as a prophylactic by radiologists.

X-ray sickness successfully treated with liver extract intramuscularly, from 1 to 4 ml. (1 ml. usually sufficient). The effect lasts 2 days.—J. H. D. Webster, *Brit. med. J.*, i/1934, 15.

Pentobarbital sodium is valuable for controlling roentgen sickness. The drug is administered in the form of a suppository in doses of 3 grains—large doses cause too great a sedative effect. Of 175 patients so treated 61·1% received complete relief from sickness, 18·3% had only moderate nausea, 16·9% had nausea with slight vomiting, and 9·7% had no relief.—W. C. Popp and M. W. Binder, *Radiology*, 1937, 28, 211.

Effects of Radiations on Normal Tissues

Skin. After a sufficient dose of radiations has been applied to the skin, an erythema or reddening is observed. This reddening appears only after a latent period of several days has elapsed. The latent period varies with the quality of the ray, being shorter with the softer rays, i.e., those of longer wave-length, and longer with the harder, rays, i.e., those of shorter wave-length. The minimum latent period is a few days, while the maximum may be 3 or even 6 weeks. The erythema varies with the dose applied, from a faint pink coloration to a dark bluish-red, and its limits follow exactly in shape and extent the area to which the rays have been applied. The appearance of the erythema is accompanied by epilation, but unless very large doses have been given so as to produce destruction of the hair follicles, regrowth of the hair is constant. The erythema passes off after a few days and is followed by pigmentation and by desquamation of epithelium. With larger doses, blisters, bullæ and even deep ulcers may be produced; the latter are extremely painful, show little tendency to heal, and may even prove fatal. Late changes in the skin may occur after intensive irradiation, even after an interval of months or years. These include telangiectases and ulcerations, which may be very intractable. Permanent damage to the skin, in the form of thickening and abnormalities of pigmentation, is not uncommon.

The physical dosage required to produce these various degrees of reaction is so constant that in the past the erythema has been made to serve as a unit of dosage. This was known as the "Erythema Dose" (E.D.) or "Unit Skin Dose" (U.S.D.), and for many years before the introduction of the international physical unit, it provided a valuable and reliable unit of dosage.

Blood. All the formed elements of the blood are affected by radiations. Of these the leucocytes are most affected, especially the lymphocytes. The red cells and hæmoglobin are only severely affected in cases in which some degree of anæmia exists at the commencement of the radiation treatment.

Mucous Membranes. The mucous membranes of the mouth, throat, intestine, bladder and rectum, may all undergo inflammatory changes after intensive irradiation. These changes present the ordinary characteristics of inflammations affecting these various organs. They are frequently produced in the treatment of malignant disease, and if the dosage has been correctly gauged they are only transient.

In mucous membrane the changes with either X-radiation or radium are essentially the same, but instead of moist desquamation (as in the case of the skin) a yellow membrane forms over the part treated. Mucous membrane appears to regenerate more easily than does skin and to tolerate a higher dose. When external radiation is given for the treatment of lesions of the mouth and throat the formation of this yellow membrane is usually regarded as a sign that a sufficient dose has reached the epithelium of the cavity.—M. C. Tod, *Lancet*, ii/1936, 702.

Eyes. The observation of eyes which have been exposed to radium irradiation of destructive intensity has demonstrated a series of morbid changes leading progressively to radium necrosis of the cornea and loss of the eye. When treatment of neighbouring malignant tumours is by interstitial irradiation these changes can be reduced by adequate screenage of the radium needles. After mass irradiation by a large quantity of radium at a distance the damage to the eye is negligible.—P. Martin, *Brit. med. J.*, i/1937, 654.

Generative Organs. Sterilisation may be produced in both male and female as a result of exposure to X-rays or radium, either for therapeutic purposes, or from accidental exposure over a long period in the course of employment. In either type of case, the condition is curable, at least in a proportion of cases.

Lungs. Severe damage to the lungs can be caused by X-rays. This damage takes the form of an acute pneumonic condition, or a chronic fibrosis with bronchiectases. These injuries were formerly fairly common after the treatment of malignant disease in the breast, but with the use of modern technique in treatment they are now rarely seen.

Nervous System. The nervous systems, both central and peripheral, are very insensitive to X-rays, and are not appreciably affected by clinical dosage.

The Treatment of Radiation Effects. For the erythema of the skin little treatment is required. Irritation may be allayed by a simple starch powder during the treatment, and by a zinc oxide, bismuth and starch powder after the treatment. Preparations containing heavy metals must *not* be used during the treatment, since the secondary radiations produced from them may cause severe damage. For blisters and small bullæ, Cycloform ointment 5% may be employed; or, when larger areas of skin are involved, flavine in paraffin 1/4000 may be used, but spirit must not be used in making up this preparation.

Recent experience has shown that all the degrees of X-ray reaction, even when the epidermis has become denuded, heal most rapidly when left exposed to the air and without any medication whatsoever. Where, however, the affected skin cannot be exposed to the air, any non-irritating, slightly antiseptic, salve may be applied, but this should only be in a very thin layer, just sufficient

to prevent adhesion of dressings and no more. Plastering the area with fatty preparations is very likely to delay healing.

X-ray or radium burns are satisfactorily treated by the following preparation: proflavine 4 4/5 gr., glycerin suppository mass 375 gr., distilled water to 10 fl. oz. Dissolve the proflavine in the water, making a 1 in 1000 solution; warm and add the glycerin suppository mass; when dissolved, strain through gauze. Apply from an all-glass spray. The preparation is antiseptic and gives a flexible crust of even thickness which forms its own dressing.—P. P. Cole and M. Lederman, *Lancet*, ii/1941, 329.

Treatment of Late X-ray Effects. Late X-ray changes in the skin are now very uncommon. Telangiectases may occur from 1 to 3 years after X-ray treatment (less frequently with radium). Other effects are atrophy of the skin, thickening of the skin, skin cracks, benign or malignant neoplasms, late ulcerations, and acute inflammation of the subcutaneous tissues; the treatment of the last two conditions is complete rest and protection of the part, even immobilisation in plaster—in the early stages a vaccine from a culture of the organisms might give rapid recovery. Telangiectases are sometimes removed by high frequency or the static machine; cracks in the nails yield to a cellulose solution; warts can be treated by paring or rubbing down with emery paper.—*Brit. med. J.*, ii/1932, 1116.

X-ray Dosage. Various methods have been used for the measurement of X-rays. Of these all are now obsolete with the exception of the ionisation methods. The latter depend upon the effects of X-rays on the electrical conductivity of the air, the air becoming ionised and consequently conductive of electricity to a degree which is dependent upon the intensity of the source of the radiation.

The method devised by Dr. Sievert, Director of the Physical Laboratories of Stockholm, Radium Hemmet, consists in the use of small spherical electric condensers, charged from a suitable source, which are introduced into body cavities, the charge being determined before introduction and after exposure to the radiations *in situ*. The use of a delicate electrometer is entailed, but the individual condensers, after withdrawal, can be packed and sent to the physical laboratory for measurement.—*Brit. med. J.*, ii/1932, 1062.

One other method of measurement of X-rays may be mentioned, as it is still sometimes used in this country. It depends upon the effect of X-rays in changing the colour of barium platinocyanide from apple-green to russet-brown, this change occurring after a given dose of X-rays has been administered. The barium platinocyanide is coated upon small discs of pasteboard which can be exposed at a given distance from the source of the X-rays. This was one of the earliest methods of X-ray dosage measurement to be introduced, and is associated with the names of Sabouraud and Noiré.

The modern unit of X-ray dosage is the international "r" unit. The value of this unit in terms of biological effect varies somewhat with the method of measurement and the quality of the ray. With rays produced at 180 kv. and a 0.5 mm. Cu filter, the Erythema Dose is equivalent to 800 "r."

Other units that may be mentioned are the "H" unit (Holznecht) and the "B" unit (Sabouraud and Noiré), but these are only of historical importance.

Complete series of estimations of X-ray and radium dosage at various depths in the body under various conditions have been prepared, and are used in the treatment of deep-seated disease.

A very small margin exists between the dose which will determine a cure and the dose which will provoke an injury. Daily examination of patients is necessary; modification of the normal tissues and of the general condition by X-ray treatment sometimes appears so quickly that it is often necessary to diminish the daily dose or the size of the fields in the course of treatment. There is no fixed method of treatment but a simple clinical treatment for each individual patient and for the special type of tumour.—Henri Coutard, *Lancet*, ii/1934, 1.

Technique. X-rays of all available qualities are used in treatment. For the treatment of skin diseases, soft rays are used produced at 70 kv. to 100 kv. with no filter or with a light filter, e.g., 1 or 2 mm. of Al. In the treatment of conditions which are fairly superficial but which still require a ray of some penetration, higher kilovoltages (e.g. 150 kv.) with a heavier filter (e.g. 5 mm. Al) are used. The effect of the filter is to absorb the unwanted, less penetrating, portion of the beam. In the treatment of deep-seated lesions and of most cases of malignant disease, the hardest rays obtainable are used, produced at 200 kv. or more, filtered with 1 mm. or more of Cu. In these conditions, hard rays are used not only because of their penetrating power, but also because it is believed that the shorter wave-lengths have a more destructive effect on the cancer cell.

Recently X-ray machines have been developed in both this country and in America, capable of working at a million volts. The first in this country was installed at St. Bartholomew's Hospital in 1937. Methods of treatment at these high voltages are still in the experimental stage.

Short-distance X-ray Therapy, or Contact Therapy. This is a method of X-ray therapy which has recently been introduced for the treatment of superficial and surface lesions, both malignant and non-malignant. In this method, the anticathode, that is, the source of radiation, is brought so close to the patient that it is almost in contact with the skin. For this purpose a special single-ended tube has been designed in which the anticathode end can be kept at earth-potential, thus obviating the danger of shock. Many advantages in use result from the fact that the operative end of the X-ray tube is free and at earth-potential. The most important of these is that the tube can be inserted into body cavities or into operation wounds, and the X rays applied directly to a lesion. Disadvantages of the method are that only soft rays can be obtained (up to the present), and that the depth efficiency of the beam is very low. In the five years or so, however, in which the method has been under trial, it has tended more and more to replace the use of surface radium for the treatment of superficial lesions. Contact therapy has already become the accepted method of treatment for certain types of rodent ulcer, epithelioma, naevi, keloid scars, and certain superficial malignant diseases. (See Chaoul and Adam, *Strahlentherapie*, 1933, 48, 31; Morison, Hugo and Mayneord, *Brit. med. J.*, ii/1935, 783; Van der Plaats, *Brit. J. Radiol.*, N.S., 1939, 138, 353.)

Treatment by Radium. The radium is used in the form of the bromide or sulphate, but the strength of the applications is expressed in terms of radium element, so as to procure uniformity. It is put up in metal containers which also act as filters. The thickness of the containers is in the great majority of cases sufficient to remove the alpha and beta rays, leaving only the gamma rays for the purposes of the treatment, although beta rays are still occasionally used in treatment. A thickness of 0.6 mm. of platinum is sufficient for practical purposes to remove the beta radiations. Thicknesses of 1 mm. or even more are,

however, sometimes used, the effect of which is to remove part of the softer or less penetrating gamma radiation.

Radium Containers in current use comprise tubes, needles, plaques and cells.

Tubes. These are of platinum, hermetically sealed, usually 1 mm. thick. Any additional screenage can be added by enclosing in capsule screens. A 10 mg. tube is often of about the following dimensions: total length 20.7 mm., external diameter 2.95 mm., length 15 mm. Tubes of all sizes are, however, used.

Needles are cylindrical, with an eyelet hole at one end and either a conical or triangular trocar point at the other. They are usually of platinum, with a percentage of iridium for strength, 10% for the body of the needle and 25% at the point. The thickness of wall varies between 0.5 mm. and 1 mm. and even more. (See also Screens *infra*.) By embedding a number of these needles containing, e.g., 1 to 3 mg., cross-fire effect is produced. "*Linear intensity*" is a new factor, conveying the amount of radium per unit length of the needle. Some hold that 0.6 mg. per cm. length of needle is a safe maximum. Standard dimensions are now in use, e.g., for a 1 mg. platinum needle: total length 26.5 mm., external diameter 1.6 mm., length of chamber 15 mm., eyelet 5 mm., point and screw 6.5 mm.

Needles can be converted into tubes for use in cavities, and into plaques for surface use, by means of suitable applicators.

Needles are now often made by packing standard small platinum cylinders into an external platinum case. This ensures more even distribution of the radioactive materials and greater accuracy of amount. Materials having a mean atomic weight about equal to that of copper give best secondary β radiation.

For holding needles in position for surface irradiation so that the radium is at a constant distance from the skin, e.g., of the neck, some medium is necessary which can be moulded to the part. The material most commonly used for the purpose is *Columbia wax*, containing beeswax 100 g., hard paraffin, m.p. 62°, 100 g., pine sawdust 20 g., melted on a water-bath, and poured into trays of the required depth. The sawdust sinks to the bottom, giving a finely granular lower surface which is applied to the skin. (See Ward and Smith, p. 164.)

Cells are similar to tubes, but generally without an eyelet, and may be used for minute amounts for building up applicators of desired radium content.

The term "**Applicator**" is now employed to cover various devices holding the container in therapeutic use.

Sheath Needles are similar to ordinary needles, but the points are screwed into the shaft and hence removable. One, two, or more, "removable cells" may be inserted.

Flat Applicators for Needles are of brass or German silver. These have windows and are made for holding 2 to 6 needles.

Needle Introducers, Prostatic Applicators, Uterine Sounds and Applicators for Antrum, Rectal, Vaginal and Oesophageal use are also available.

Screens of Lead of various thicknesses are used, e.g., 1 mm. thick, covered with rubber, allow practically only γ rays to pass.

Lead $\frac{1}{16}$ to $\frac{1}{8}$ mm. permits the hard beta rays to pass as well as the gamma.

Silver 1 mm. absorbs 99% of the hard β rays and Brass 1.3 mm. absorbs the same amount. When the aim is to utilise β rays, the duration is relatively so short that the γ ray effect is negligible. Screens are necessary to cut off the β rays because of their greater action on the superficial structure.

Platinum $\frac{1}{8}$ mm. screens off 99.9% of the primary β rays and enables the use of all γ rays.

Gamma radiation acts by dissolution and absorption, while beta radiation acts by destruction through necrosis, with consequent suffering to the patient. The importance of adequate screening.—Sir C. Gordon-Watson and Stanford Cade, *Lancet*, i/1929, 634.

The therapeutic effect of the gamma rays is due to the high speed electrons they set free from the tissues, chiefly as "recoil electrons."

The methods of radium therapy are divisible under three headings:—(1) Interstitial therapy; (2) Short distance surface therapy; (3) Telecurie or "Radium Bomb" therapy.

The introduction of a scientifically based and accurate method of radium dosage (Paterson and Parker, *Brit. J. Radiol.*, N.S., 1934, 7, 82) has revolutionised the practice of radium therapy (apart from telecurie therapy). The object of the method is to provide data to secure a uniform distribution of radiation throughout the volume of tissue being irradiated. For this purpose the precise distribution of radiation around the various types of radium sources has been measured, and a scheme drawn up giving the arrangement of sources for every type of case that may be met with in practice.

In *surface therapy*, the tubes or needles are arranged on a suitable carrier which is devised so as to hold them at a stated distance from the skin surface. Metal plaques which were used formerly are now very rarely used. As has been stated above, the field for surface radium therapy has been much contracted by the introduction of the Contact or Short-distance Therapy method.

In *interstitial therapy*, the needles are inserted into the tissues in planes and patterns which can be determined from the Paterson and Parker scheme.

In *telecurie therapy* large masses of radium (1 to 5 grammes or more) are used at a distance of several centimetres from the surface.

In 1933 a special Radium Conference appointed by the Royal Colleges of Physicians and Surgeons reported that there was a field of usefulness for radiation from massive units of radium and recommended that a radium unit containing not less than 5 g. of radium element be established. 5 g. of radium having been loaned by the Union Minière de Haut-Katanga for the purposes of this research, with the promise of a further 5 g. if necessary at a later date, a representative governing body of seven members was formed to organise the research, the location being the London Radium Institute (see *Brit. med. J.*, i/1933, 28; i/1933, 121).

Post-radiation care. Full therapeutic effect is not seen till 2 months after treatment and during that time there may be lassitude and anorexia, and, when large, highly cellular tumours are resolving rapidly, considerable constitutional disturbance. Transient oedema of the skin develops a week after completion of treatment, developing into a well-marked erythema, vesication, and even superficial ulceration, which does not subside for 6 or 8 weeks, and during this time a soothing local dressing must be used. After treatment of the mouth and upper air passages there is oedema and inflammation of the mucous membrane during the week following, the tongue later becoming coated with a yellowish diphtheroid membrane, under which healing takes place. During this time (about 8 weeks), the mouth should be irrigated at least six times a day, and smoking, condiments and sauces should be avoided. After-care of carcinoma of the cervix should be undertaken on the same general principles. If efficient douching is not undertaken an adhesive vaginitis forms in the upper half of the vagina.—Roy Ward, *Practitioner*, ii/1933, 513.

DISEASES TREATED BY RADIATIONS (X-RAYS AND RADIUM)

(1) *Non-malignant*

ACNE. Good results are obtained by X-ray treatment, small doses being applied weekly.

ACTINOMYCOSIS. Good results are obtained by X-ray treatment. In the localised conditions, large doses of X-rays may be used, applied over a comparatively short time, and results are fairly quickly obtained. In the extensive, hitherto hopeless, conditions, however, the essential feature in the treatment is the application of repeated very small doses of X-rays at intervals of several

days over a period of many months. It is a very tedious method of treatment, and improvement is not seen for several weeks but when it occurs it is steady and continuous and the results are very gratifying.

ACUTE INFLAMMATORY CONDITIONS. Resolution of these conditions is often assisted by the application of small doses of X-rays at intervals of several days.

Of 855 cases of inflammatory affections, including boils, erysipelas, anthrax, sinusitis, mastitis, orchitis, epididymitis, otitis media, etc., treated with small doses of X-rays, 76% were cured without operation, 19% were doubtful, and 5% were failures. Dosage recommended: 10 to 20% of the erythema dose and exposure limited to a few minutes at intervals of 3 to 4 days. Earlier failures probably due to excessive dosage.—A. Plichet, *Pr. méd.*, Mar. 4, 1933, 349.

AMENORRŒIA. Good results have been claimed from the application of small doses of X-rays to the ovaries and pituitary gland.

ARTHRITIS. Considerable relief may often be obtained, especially when the disease is recent and is mainly peri-articular. The rays are applied weekly in moderate doses.

BRONCHIECTASIS. Roentgen therapy in moderately large dosage as the sole method of treatment for chronic secreting bronchiectasis is feasible and successful, resulting in great symptomatic improvement in a considerable proportion of cases. Follow-up examination over a period of two years in those cases in which there has been improvement has shown no recurrence of symptoms with infections of the upper respiratory tract.—M. Berck and W. Harris, *J. Amer. med. Ass.*, i/1937, 518.

CORNS and CALLOSITIES. Excellent results are obtained by the application of large localised doses of X-rays or gamma rays.

ECZEMA. Sub-acute and chronic cases are suitable for X-ray treatment. Complete relief may be obtained, but recurrence is common. One or two sittings, with moderate dosage, may be all that is required.

EXOPHTHALMIC GOITRE. Good results are obtained by the application of moderate doses of X-rays to the neck weekly.

X-ray therapy should be given a trial in the large majority of cases, the results considered at 3-monthly intervals, and those not showing adequate improvement sent for surgical treatment. In patients who, for financial or other reasons, cannot face more than a short absence from active life, an operation seems the treatment of choice.—C. S. D. Don, *Brit. med. J.*, i/1934, 748.

It is equally an exaggeration to say that either X-rays or surgery is the treatment of choice. Neither method effects a cure but both have their place. In favour of surgery however, it should be said that the results are obtained in a fraction of the time required by radiological treatment.—G. Keynes, *Brit. med. J.*, i/1933, 673.

HODGKIN'S DISEASE (LYMPHADENOMA). This disease is incurable, but the local glandular swellings respond well to moderate doses of X-rays; although recurrence is the rule, life may be prolonged for several years.

In most cases of Hodgkin's disease and lymphosarcoma, rays of medium wave-length are distinctly preferable. Lymphoid cells are so sensitive to irradiation that a moderate quantitative dose of rays generated at 135 to 140 peak kilovolts and filtered through 4 or 6 mm. of platinum (depending on whether the nodes irradiated are in the subcutaneous tissues or in the mediastinum or abdomen) is sufficient to induce marked regression of the hyperplastic nodes. Exposure of many areas to rays of this quality may be repeated several times without undue risk, when the intervals between courses of treatment are a matter of months. The effect of treatment is rapid reduction in size of the enlarged lymph nodes in the irradiated regions, dyspnoea is often relieved within a few days, and gastro-intestinal disturbances rapidly subside. Treatment may not be so effective in the advanced stages. Even if the treatment does not notably prolong the life of the average patient, it controls the manifestations of the disease and relieves the symptoms.—A. U. Desjardins, *J. Amer. med. Ass.*, ii/1932, 1233.

HYPERTHYROIDISM. Its use is indicated in the following selected types: in children and adolescents, except in those with nodular goitres and cardiac complications; in mild to moderately toxic adults without visceral complications or marked loss of weight, and who have a diffuse soft or elastic goitre with symptoms of sympathetic nervous disturbances. The treatment is further indicated where the surgical risks are great or where the patient refuses operation, or in patients

with recurrent post-operative hyperthyroidism which cannot be controlled by iodisation. In the presence of a progressive increase in the severity of the disease irradiation should be abandoned and the patient prepared for thyroidectomy.—J. H. Harris and E. Rose, *Amer. J. Roentgen.*, Nov. 1936, 610.

INFLAMMATION. Small doses of X-rays are of value in a wide variety of inflammatory conditions. The proportion of successful results in bursitis is 52%, in carbuncles 45%, in cellulitis 78%, in furuncles 72%, in post-operative parotitis 52% and in warts 98%. (A case is only called successful if it is relieved in a considerably shorter time than is usual when radiotherapy is not employed.) The technique is simple, and the doses so small—from 100 to 200 r daily for three to five days—that there is no risk of radiation injuries.—Pendergrass and Hodes, per *Brit. med. J.*, ii/1941, 700.

KELOID SCARS. Excellent results are obtained by the application of full doses of X-rays or radium, sharply localised to the region of the scar.

LEUKÆMIA. The chronic forms of this disease respond well to X-ray treatment applied to the spleen, and the blood-picture may be restored to an approximately normal condition. Recurrence is the rule after a variable number of months, when temporary success may again be obtained by repeating the treatment. The disease is invariably fatal, although its course may be prolonged for several years.

Chronic leukæmia will respond satisfactorily to much smaller individual and total doses of roentgen rays than those commonly employed. Any individual dose which produces nausea or a feeling of increased toxicity is probably an excessive dose.—A. H. Dowdy and J. S. Lawrence, *J. Amer. med. Ass.*, i/1941, 2827.

MENORRHAGIA. This condition can be cured by X-rays applied to the ovaries in sufficient doses to cause sterilisation. The best subjects for treatment are those in whom menorrhagia occurs at the menopause, when the X-rays are used to expedite the natural atrophy of the ovarian cells. Except in cases of extreme urgency, X-rays should not be applied to the ovaries during the child-bearing age.

Radium is of great value in uterine hæmorrhage. One application of radium element to the interior of the uterus for 24 hours.—P. C. Fenwick, *Brit. med. J.*, ii/1929, 455.

MYASTHENIA GRAVIS. X-rays have recently been recommended in the treatment of this condition. It has been found that in association with myasthenia gravis the thymus gland may be enlarged, and the irradiation is directed to the thymus gland. Successful results have been reported in a number of cases.

MYOMA UTERI. This condition is treated on the same lines as those for menorrhagia.

NÆVI. Radium treatment is "One of the most successful of all medico-surgical treatments." Important general principles are: (1) To treat the patient at as early an age as possible, and at any rate in the first year of life. (2) The effect of the treatment is very slow and continues for a year after the application, so the treatment must not be repeated too soon. (3) Caustics or carbon dioxide snow must not be used as they leave scars which show after the case is cured by radium. (4) In all cases, heavily screened (or filtered) gamma rays must be used. (5) The dose must be such as to produce only a moderate erythema. (6) In some cases the assistance of a plastic surgeon is invaluable for removal of redundant scar tissue.—N. S. Finzi, *Brit. med. J.*, ii/1935, 591.

In recent years, contact therapy has tended to replace surface radium in the treatment of these conditions, and the results are at least equally good.

NEURALGIA. Relief is often obtained, especially in the trigeminal and post-herpetic varieties, by the application of weekly doses of X-rays to the Gasserian ganglion and the posterior nerve roots respectively.

OTITIS MEDIA. Fifty consecutive cases of otitis media (31 acute, 8 subacute, and 11 chronic) were treated by roentgen therapy over the ear and mastoid area. The treatment was consistently followed by relief of pain, increased discharge, improved hearing and improvement of general condition. The treatment shortens the course of the disease and prevents complications. No deleterious effects were observed. The technique advocated is 100 to 110 kv., 16 inch distance, 2 mm. aluminium, 5 ma., 5 minutes; tube output 14.3 roentgens per minute. The total dose is 71.5 roentgens measured in air; usually a single dose is sufficient.—J. H. Lucinian, *Amer. J. Roentgenol.*, 1936, 36, 946, per *Canad. med. Ass. J.*, i/1937, 654.

PNEUMONIA. Of 104 cases of lobar pneumonia, including cases of infection due to types I, II and III pneumococci, as well as to the higher types of pneumococci and other bacteria, treated with roentgen therapy, only 5 patients died. In the treatment of type I infections roentgen therapy seemed to give better results than serum. The roentgen therapy employed was similar to that used for carbuncles, furuncles, etc. The only contraindication appeared to be definite leucopenia.—E. V. Powell, *Canad. med. Ass. J.*, i/1938, 19.

PRURITUS. The general conclusions of leading radiologists and dermatologists are that in localised forms of pruritus X-rays give distinct relief in from 60 to 90% of cases and cure in about 50%, but fail in diffuse pruritus. Caution is necessary; if no response follows the first treatment proceed cautiously; if no response follows the second, discontinue; further, "proceed with extreme caution when patients have already had X-ray or radium treatment."—Lord Horder, *Lancet*, ii/1935, 287.

PSORIASIS. This is often successfully treated by X-rays, especially in cases which are not of long standing. Recurrence is frequent, but a complete cure may be obtained. Moderate doses are applied at intervals of several days.

RINGWORM OF THE SCALP. X-rays offer the only means of a complete cure in this condition. Sufficient dosage must be applied to the scalp at one sitting to produce complete epilation.

SYCOSIS. In this condition also, a cure may be obtained by producing complete epilation.

SYRINGOMYELIA. Some good results have been claimed for weekly applications of moderate doses of X-rays.

THROMBOPENIA. Roentgen radiation constitutes an exceedingly valuable and possibly a specific therapeutic agent when applied over the spleen in primary or uncomplicated thrombopenia with hæmorrhage either with or without purpuric skin manifestations. Report of seven cases successfully treated.—H. Rudisill, *J. Amer. med. Ass.*, ii/1936, 2119.

TUBERCULOSIS. When localised to glands, joints or skin, this condition may often be helped by the application of small doses of X-rays at weekly intervals.

TUBERCULOUS MENINGITIS. Five cases in children completely cured by deep X-ray therapy, employing 162 kilovolts, 4 ma., with 0.25 of zinc and up to 3 mm. aluminium filter, 150 to 200 r units, with a focal distance of 34 cm., applied to the base of the neck, and to the forehead and temporal regions, three to four applications being made with 1 to 2 days between the first two, and 3 to 4 between subsequent applications.—Prof. Z. von Bokay (Hungary), per *Lancet*, i/1932, 894.

(2) **Malignant.** A malignant growth or cancer is a growth which if left untreated steadily extends, pressing upon and eating away the structures with which it comes in contact and eventually destroying life, either by the local damage it causes or by the formation of one or many secondary growths in other regions of the body. The two main varieties of cancer are *carcinoma*, arising from epithelial or covering tissues, and *sarcoma*, arising from connective tissues. Many varieties of carcinoma and some of sarcoma can be destroyed by adequate irradiation, either by X-rays or by radium. Often, however, even though the local growths can be destroyed, secondary deposits are subsequently discovered and these may be multiple and lead to the death of the patient. In many cases the best result is only obtained by a combination of X-rays and radium.

When X-rays are used in the treatment of cancer, only the hardest obtainable rays (i.e., the shortest wave-lengths) should be employed. This applies equally whether the growths are superficial or deep. As a general rule, the hardest rays obtainable are produced at 250,000 volts, and it is important that filtration should be adequate (not less than 1 mm. of copper).

Radio-sensitivity of malignant growths. (1) *Highly radio-sensitive* (growths that can be made to disappear by a dose of radiations producing little or no damaging effects on normal tissues)—(a) small, round-celled and lympho-sarcoma; (b) seminoma; (c) some carcinomas of the breast; (d) some carcinomas of the ovary; (e) certain cerebral tumours—medulloblastoma. These should invariably be treated by external radiation, X-rays or telecurie therapy, preferably X-rays. (2) *Moderately radio-sensitive* (growths which can only be destroyed by a dose of radiation approaching fairly closely the maximum tolerance dose of the healthy tissues)—(a) round-celled sarcoma; (b) endosteal and chondrosarcoma; (c) some periosteal sarcomas; (d) most carcinomas of the breast; (e) carcinoma of the prostate; (f) carcinoma of the bladder; (g) certain palatal, pharyngeal and laryngeal growths—lympho-epithelioma; (h) hypernephroma; (i) carcinoma of the cervix; (j) of the corpus uteri; (k) of the ovary; (l) of the thyroid gland; (m) of the lip; and (n) of the skin. Interstitial radium is best for this group wherever possible, except in extensive (carcinoma of skin) or inaccessible areas, when X-rays, or X-rays followed by radium, may be used. (3) *Radio-resistant growths*—(a) carcinoma of rectum; (b) oesophagus; (c) tongue; (d) many pharyngeal carcinomas; (e) some carcinomas of the breast; (f) and of the ovary; (g) carcinoma of the bronchi; (h) certain metastases in cervical glands, especially arising from carcinoma of tongue; (i) spindle-celled sarcoma and fibrosarcoma. In these interstitial radium is the treatment of choice, but the whole extent of the growth must be thoroughly barraged and with full dosage. X-rays rarely achieve more than some degree of palliation.—W. M. Levitt, *Brit. med. J.*, ii/1933, 678.

A Method of Spacing Radiation in the Treatment of Tumours. The method is based on two facts. The first is that cells under anærobiosis are more resistant to irradiation than cells well oxygenated. The second fact is that radiation, through the action on capillaries and small blood vessels, interferes with the circulation of the tissue or tumours radiated, so much so that when an animal bearing two tumours, one radiated and one non-radiated, is inoculated with Indian ink a few days after the application of radiation it is found that the ink enters the normal tumour but fails to gain entrance into the radiated tumour. It follows that during the interference with the circulation following radiation, tumours must be radioresistant and that they will not again become radio-sensitive until this damage to the blood vessels has been recovered from. Hence the possibility arose that a system of spacing irradiation which took account of these factors might in certain cases prove both practicable and justifiable in the treatment of patients suffering from malignant disease. For this purpose it would be necessary to detect changes in the circulation of the tumours during treatment. Frequent measurements of the growth of tumours in patients will therefore indicate, by slowing or cessation of growth, times of vascular damage; and by recrudescence of growth, times of recovery from vascular damage; and so also times of radioresistance and of radiosensitivity. Treatment is administered either continuously, or at short intervals until the tumour shows an initial decrease in size; it is then discontinued until the measurements show an increase, when the process is repeated. To obtain the best results some measure of the radiosensitivity of the skin and normal tissues is required.—J. C. Mottram and J. Morton, *Lancet*, ii/1936, 672.

The regressing of a malignant new growth after irradiation is the result of a complex process and is not due to a direct lethal effect of the irradiation on the malignant cells. The process is initiated when the malignant cells have been damaged by irradiation. This damage elicits a reaction of repair beginning with an invasion of the tumour by macrophages which become gradually transformed into fibroblasts so that the tumour is gradually replaced by young cellular connective tissue. Malignant tissues in general are not more radio-sensitive than normal tissue in general. There are malignant growths which are very radiosensitive and others which are very radioresistant, and the same applies to normal tissues. The radiosensitivity of malignant cells is not a fixed inherent property but can be varied within wide limits. In the absence of oxygen malignant cells become very radioresistant, while their radiosensitivity can be greatly enhanced by inhibiting respiration either by HCN or by cold. In all methods of radiotherapy, therefore, involving repeated application of radiation it is essential that the doses given are so small that they do not permanently damage the vasculo-connective tissues surrounding the tumour, for if this happens the blood supply to the tumour is interfered with and the malignant cells pass into a stage of partial anærobiosis which makes them radio-resistant. This induced radioresistance is a well-known clinical phenomenon.

It is, however, not due to a direct effect of radiation on the malignant cells but results from damage to the blood supply. The damage inflicted upon cancer cells by sub-lethal doses of radium is one which persists for some time but from which the cells can recover. The period of recovery, however, for malignant cells is, for very small doses, very much longer than in normal tissues, and thus by applying very small doses at suitable intervals it becomes possible to produce a cumulative effect in a tumour with a non-cumulative, or a much less cumulative, effect on the skin, thus bringing about a selective action on the malignant tissue. Experiments on mice with spontaneous mammary tumours, in which the tumour had been removed by operation and autoplasts had been made in the flanks have shown that in those animals in which a single large dose had an effect the repeated administration of small doses was even more effective. It is, therefore, justifiable to conclude that the method is theoretically sound and should be given a trial in the radiotherapy of cancer in man. The essential features of the method advocated on the basis of the experimental investigation are the use of very much smaller doses at intervals much longer than is now the practice. The method has the advantage that it produces a more selective effect on the tumour and thus avoids or delays the skin reactions which ordinarily limit the total dose of radiation which can be applied. The suitable doses and intervals of time for man have to be determined by clinical experience, using as a guide the biological effects produced on the tumour and bearing in mind the importance of avoiding damage to the skin and blood vessels surrounding the tumour. The method has the further advantage that if it fails to bring about the complete disappearance of a tumour it makes it possible to induce in the remaining cancer cells a "dormant condition" in which the malignant cells, while retaining their viability, fail to grow, and can be maintained in this condition by continued irradiation with small doses at long intervals, so that the disease, while not cured, is arrested.—W. Cramer, *Lancet*, ii/1936, 668.

CANCER OF THE TONGUE, FLOOR OF MOUTH, AND TONSIL may be treated by interstitial radium therapy or by telecurie, according to the special indications of the case. Where enlarged glands are present, the consensus of opinion seems to favour block dissection rather than irradiation of the gland. (See Duffy, *Amer. J. Röntgen.*, 1938, 33, May 5th.)

EXTRINSIC CANCER OF THE LARYNX has so far failed to yield results with any method of radiation treatment.

INTRINSIC CANCER OF THE LARYNX, on the other hand, does well with either X rays or radium or telecurie therapy.

Of 45 cases of lymphosarcoma of the mouth and tongue treated by X-rays, 17% were alive both after 5 years and after 7 years. Of 46 cases of epithelioma of the tonsil and soft palate, 28% were alive after 5 years and 17% after 7 years. Of 77 cases of epithelioma of the larynx 28% were alive after 5 years and 27% after 7 years.

A voltage of 180 to 200 kv. used, a filter of 2 mm. Zn, 3 mm. Al, 1 cm. wood; a milliamperage of 4 and a distance of 50 to 60 cm. A constant potential machine used and the dose measured in "r": for radio-sensitive tumours the depth-dose measured from 3500 to 5000 r and for radio-resistant tumours 4000 to 5000 r or more. Results exactly measured by biological consequences—radio-epithelitis, involving complete destruction of the germinal layers of the mucous membrane and production of a false membrane, destruction being complete in 13 or 14 days, and the lesion healing on the 26th day; and radio-epidermatitis, involving loss of epithelial layers and denudation of the dermis, appearing in 26 to 28 days, with healing 15 days later.

Cure of cancer by X-rays possible, but very difficult and still dangerous.—Henri Coutard (Fondation Curie, University of Paris), *Lancet*, ii/1934, 401.

CANCER OF THE ŒSOPHAGUS has recently yielded very encouraging results treated by X-rays by a method introduced by Levitt (*Proc. Roy. Soc. Med.*, 1934).

CANCER OF THE STOMACH AND INTESTINAL TRACT have so far not proved amenable to radiation treatment.

CANCER OF THE SKIN, whether epithelioma or rodent ulcer, gives good results with radiation therapy. High voltage, X-ray therapy, contact therapy, surface radium, and interstitial radium, all have their place in the treatment of these conditions. Nowadays, the smaller lesions are nearly always dealt with by contact therapy, while the larger ones do well with high or medium voltage

X-ray therapy, or with radium therapy. The results are better than in any other form of malignant disease, and in the absence of bone or cartilage involvement are very nearly 100% of cures. Large doses are required, but as they can be localised to the region of the growth, there is little or no constitutional reaction.

CANCER OF THE LUNG yields a very small percentage of palliative results.

CANCER OF THE WOMB (CERVIX AND BODY) is successfully treated by a combination of X-rays and radium.

The "Stockholm technique," with some modifications, is that generally used in this country for treatment of cancer of the cervix. It consists of placing radium (roughly 40 to 50 mg.) into the cervix and body of the uterus and into the vagina (60 to 70 mg.) on three occasions of 22 hours each with an interval of a week between the first and second and between the second and third, the filter used being 1.5 mm. platinum with a special pessary for holding the radium.—M. Donaldson, *Brit. med. J.*, i/1934, 547.

CANCER OF THE PROSTATE AND TESTIS IN THE MALE gives a proportion of good results when treated by X-rays.

Some **TUMOURS OF THE BRAIN** are amenable to X-ray treatment.

In the case of **SARCOMATA**, the result of treatment is determined by the variety of the growth, rather than by the site of its occurrence. Thus, lymphosarcoma is extremely sensitive to X-rays in whatever region it occurs, while a fibrosarcoma is very resistant.

SARCOMA OF BONE yields only a small proportion of good results with X-ray treatment.

GENERAL REFERENCES TO RADIATION THERAPY

(a) *Non-malignant*

Actinomycosis well treated by X-rays, and in tuberculous lesions of all kinds the greatest success has been attained—dosage must be small in order not to destroy the tubercle—in cervical glands one-sixth of an erythema dose at weekly intervals. Acne and eczemas respond well to the same dosage. In enlarged tonsils in children, X-ray results were very satisfactory. Pelvic inflammation in women, especially when due to tubercle, could be cured, and ocular inflammation did well with small doses.—J. H. Webster, *Brit. med. J.*, ii/1932, 310.

(b) *Malignant*

Results obtained with a 4-gramme "bomb" at the Westminster Hospital.

(1) Proved squamous-celled carcinoma of the buccal mucosa, floor of the mouth, palate, tonsil, lateral pharyngeal wall, pyriform fossa, and extensive carcinoma of the larynx disappeared *in toto* with complete healing.

(2) Cervical and inguinal glands secondary to squamous-celled carcinoma, fixed and inoperable, responded in certain cases.

(3) Spheroidal-celled carcinoma of the breast, unsuitable for surgery or needling; disappearance of primary growth and axillary glands; inoperable tumours retrogressed to permit of surgical removal.

(4) Bone sarcomata, in the long bones and vertebrae; made to ossify and replaced by apparently normal bone tissues.

The best form of bomb would be one containing the radium only during the period of treatment, as there is no haste on the part of the attendant to get out of the treatment room and the bomb can be placed carefully and accurately in position; it can also be much lighter as the lead upper half (required for protection of attendant making adjustments) can be removed.—H. T. Flint, L. G. Grimmett, E. R. Carling and Stanford Cade, *Brit. med. J.*, i/1934, 653.

REPORT ON RADIUM BEAM THERAPY RESEARCH, 1934-1938. In cases in which the growth is early and localised its complete disappearance can be expected. Where there is secondary involvement of the lymphatic gland in close proximity to the primary growth the enlargement of the glands can also be made to disappear. When both the primary growth and glands are in an inoperable stage in a small proportion of the patients the disease can be made to disappear and in a larger proportion distressing symptoms may be relieved. When distant metastases have already developed their growth will be progressive and inevitable even though in the region treated the growth has been destroyed. Though it is too early to make definite pronouncements it would appear that treatment of carcinoma of the mouth and throat by radium beam is at least as

satisfactory as that provided by surgery or by interstitial radium therapy. It has the further great advantage that the results are obtained without mutilation of the patient.—A. P. Wood, L. G. Grimmett, T. A. Green *et al.*, *Spec. Rep. Ser. med. Res. Coun., Lond.*, No. 231, 1938.

Protection. The increasing use of Radium Beam Therapy has suggested some experiments, leading to the conclusion that nurses and attendants should not remain for any appreciable time at distances within, say, $1\frac{1}{2}$ metres behind the bomb, $2\frac{1}{2}$ metres from the side of it, or $4\frac{1}{2}$ metres in front of it in the direct beam.—*Brit. med. J.*, ii/1934, 952.

RADON

Radon, or radium emanation, is also used in the treatment of malignant disease. Its therapeutic indications and the radiations emitted are identical with those of radium salts, but in calculating dosage, allowance has to be made for the fact that the intensity of radiation is not constant, but is continuously decreasing, falling to half its value in 3.85 days. In the complete destruction of a millicurie of radon, γ rays equivalent to 132 mg.-hrs. are emitted. The γ rays from a radium needle remain constant during a treatment, whereas the activity of the radon decreases exponentially with time. Their biological effects are not therefore necessarily equal. The emanation is filled into small gold or platinum containers; these are implanted in the tumour and allowed to remain in position for a calculated period of time. The containers, or "seeds," have a thread attached, and frequently this is fixed in position with the aid of a solution of mastic in benzene.

The seeds contain 1.5 millicuries of radon at the time of implanting, giving 200 millicurie-hours' radiation if left for 14 days. The platinum screen allows about 90% of the gamma rays to pass through, with less than 5% of beta rays. A trocar with a special cannula is used for implanting the seeds, which are implanted about 2 cm. apart. While customary to remove seeds when period of activity is over, no ill-effects have been noted where seeds have been left permanently in the tissues. Encouraging results with tumours which are difficult to treat by other methods. The initial response is more rapid than with other forms of radium therapy. The local reaction is brisk in some cases.—P. Gosse and F. E. Chester-Williams, *Lancet*, ii/1928, 323.

CARCINOMA OF THE BREAST. Radon seeds are implanted into the tissues beneath the breast in such a manner that the whole of the breast and its lymphatic drainage is exposed to a uniform radiation sufficient to destroy carcinoma cells whether in the tumour, the breast, or the lymphatics. About 100 seeds are introduced, each containing 0.5 to 1 millicurie, and the bulk of the seeds will lie in the fascia covering the pectoralis major. By inserting the rows of seeds at right angles a kind of grid of seeds is formed beneath the tumour. Irradiation by means of seeds presents immense advantages in advanced cases and elderly patients, giving results at least as good as those from amputation, with less inconvenience and no disfigurement. In early cases it may be used with considerable prospect of success. To avoid skin reaction and ulceration the whole of the breast and surrounding region should be covered with zinc and castor oil ointment, removed and replaced daily.—H. S. Souttar, *Brit. med. J.*, i/1933, 813. In hopelessly inoperable cases radon seeds are preferable to any other form of treatment.—J. K. Harper, *Brit. J. Derm.*, Nov. 1936.

CARCINOMA OF THE ŒSOPHAGUS treated by radon seeds. Of 16 cases treated, 1 was alive and well after 3 years, 2 lived for more than a year, 11 obtained

considerable temporary relief, 2 were not relieved, and 3 were able to remove gastrostomy tube. (Successful operative extirpation is a virtual impossibility, and operative mortality is in the region of 100%.)—T. B. Jobson and G. H. Steele, *Brit. med. J.*, i/1934, 233.

CARCINOMA OF THE UTRINE CERVIX. Some patients treated with X-rays and radium with no results recovered after a single application of radon. Results better where no previous treatment had been applied and where the intratumoural method of treatment is applicable. The introduction of needles is advisable only where the tumour is sufficiently large and is not closely adjacent to the bone; best results in cancer of the tongue, lip, penis, and other similar organs, but the application of masks with radon gave favourable results in other parts. The average dose for superficial application or filtration through 2 mm. of lead is 1 to 1.5 med. (millicuries détruites—decomposed radon) over 1 sq. cm. of surface, decreasing this for large areas (100 sq. cm. or more). The radon is usually left for 7 to 10 days.—M. I. Nemenow and F. S. Grossmann, *Brit. J. Radiol.*, N.S., June 1928, 187; July 1928, 245.

RELATIVE VALUES OF SURGERY AND RADIATION

In a discussion at the International Conference on Cancer, London, July, 1928, G. REGAUD (Radium Institute, Paris), said that radio-sensitivity of cancer was extremely variable. Of the two different species of cancer of the cervix uteri, epidermoid (a stratified pavement epithelioma, showing structurally the morphological changes or manner of growth peculiar to the epidermis) and glandular, cures by selective radiotherapy had been obtained only in the former, due apparently to the activity and rhythm of division on the one side, and the secretory function on the other. In addition, many factors independent of radio-sensitivity influenced the results of radiotherapy, e.g., extent of primary cancer, its distant spread, its accessibility, and the radio-resistance of the intervening tissues and organs. Ray action could be used on a small neoplasm where it was out of the question in a more extensive one.

CANCER OF THE CERVIX UTERI. M. DONALDSON (St. Barts') gave the following grounds for definitely deciding in favour of radiation as against hysterectomy—(1) the negligible mortality with radiation, (2) statistics of survival-rate in no way inferior, (3) with improved technique, more patients will seek early advice, with consequent improved results, (4) it will bring into general treatment radiotherapy in incurable cases, (5) it will encourage the younger gynaecologists to adopt a method of treatment which they will be able to carry out more successfully than the difficult Wertheim's operation. W. P. HEALY (New York): The most important determining factors in prognosis were the clinical stage of the disease and the radio-sensitivity of the tumour, and, when surgery was employed, early diagnosis and the degree of malignancy. COMYNS BERKELEY (London), gave figures relating to the radical operation. He considered that if the glands were carcinomatous the immediate operative mortality was raised from 12 to 20.6%. VICTOR BONNEY (London) thought a five-year survival period not enough—ten years should be taken before absolute cure was claimed.

CANCER OF THE BREAST. Prof. BURTON LEE (Cornell University): Irradiation in conjunction with conservative surgery had justified itself. Other speakers were in agreement with this view.

CANCER OF THE BUCCAL CAVITY. DOUGLAS QUICK (New York): Radium preferable to X-rays. Filtered radon "seeds" employed with good results. Applicators within the mouth were of no value. STANFORD CADE (London): Surgical treatment had given results so indifferent that those obtained by radium appeared brilliant. In an operable growth the choice should be local excision with the diathermy knife and subsequent irradiation of the scar.

CANCER OF THE BREAST. Results obtained at St. Bartholomew's indicate that the five-year survival rate is about the same for the two branches of treatment, being about 40% in each case; it is now decided there to combine interstitial radiation with surgery, the breast and glands being excised after radiation, and to compare the five-year survival rates with those of operation and radium respectively.

Comparison of results in the treatment of cancer of the breast tend to favour the irradiation method in preference to radical surgical procedures in all groups.—C. M. Henry, *Canad. med. Ass. J.*, ii/1937, 261.

CANCER OF THE CERVIX. The Marie Curie Hospital reports a steady improvement in the ratio of early cases. Treatment is by intercavitary radium alone.

CANCER OF THE BUCCAL CAVITY. Primary lingual growths are dealt with at most radium clinics by radium, either interstitial or by means of larger units externally; with regard to glands there seems an even balance of opinion and practice between surgical removal and irradiation. A slight improvement in oesophageal cases has followed the distribution of large doses of radon seeds (8 instead of 4 of 3 mc. each) throughout the length of the growth.—*Brit. med. J.*, ii/1934, 821.

CANCER OF THE TONGUE. The present position is that excision of the tongue has given place to radiation. A table compiled from the records of ten institutions during 1931 (none of which specialised in radium treatment) shows that fewer cases were treated by surgery alone than by radium alone, while radiation treatment of some kind entered into the therapeutic measures adopted in 67·5% of all cases.—“The Medical Uses of Radium,” *Spec. Rep. Ser. med. Res. Coun.*, Lond., No. 174, 1932; *Brit. med. J.*, ii/1932, 893.

Five-year results (Middlesex Hosp.) equal or excel those of operation, but combination of radiation with operation may double the percentage of five-year surgical cures.—J. H. Douglas-Webster, *Brit. med. J.*, ii/1932, 47.

THE TREATMENT OF METASTASES IN MALIGNANT DISEASE. The value of radiotherapy in these conditions is discussed in an article by M. C. Tod, *Brit. J. Radiol.*, N.S., May 1940. The value of the method as a palliative is indicated, especially in metastatic deposits in bone from carcinoma of the breast and other conditions, in abdominal metastases from testicular growths, in metastases in carcinoma of the thyroid gland.

ELECTROTHERAPY

THE USE OF GALVANIC, FARADIC AND SINUSOIDAL CURRENTS FOR THE TREATMENT OF DISEASE

The physiological and therapeutic effects of electricity are the consequence of certain chemical and physical changes, either ionic or thermal in character, that it brings about in the body tissues. Although there are still many gaps in the exact knowledge of the links in the chain of events which connect the application of electricity and its therapeutic results, enough is known to justify the belief that it is a rational and valuable method of treatment.

If two copper wires leading from the poles of a galvanic battery are immersed in a solution of either sodium chloride, sodium hydroxide or hydrochloric acid, without the wires touching each other, the ions, which had previously been moving about in no particular direction, now, under the influence of the electromotive force (E.M.F.) of the battery, migrate in an orderly manner in definite directions—those ions with positive charges (kations) migrate away from the positive pole (anode) and make their way towards the negative pole (kathode)—those with negative charges (anions) migrate towards the positive pole of the battery (anode).

When the positively charged kations and the negatively charged anions reach the negative and positive poles respectively, they lose their electric charges, become free unelectrified atoms and chemical combination takes place.

The ions in the tissue fluids, in which the most numerous ions are those of chlorine and sodium, can likewise be made to migrate and form new chemical compounds at the electrodes. These chemical compounds are alkaline at the negative electrode, and acid at the positive electrode, if it be made of platinum, but neutral if the positive electrode be made of zinc or copper.

Sodium hydroxide and hydroxyl ions, hydrochloric acid and hydrogen ions, zinc chloride and zinc ions, and copper chloride and copper ions are all caustics. The use of the galvanic current for the production of caustics in abnormal or unnecessary tissue in order to destroy it is a form of galvanic treatment known as Electrolysis, Surgical Ionisation or Electro-chemical Cauterisation. The active electrodes used are narrow metal rods varying in width from 0.2 mm. to 1 or 2 mm. They are inserted into tissue or introduced into channels.

Electrical currents used in the treatment of disease are the following:—

1. Galvanic.
2. Faradic (a) Primary.
(b) Secondary.
3. Sinusoidal alternating (a) Quick.
(b) Slow.
4. Combined currents $\left\{ \begin{array}{l} (a) \text{ Galvano-faradic.} \\ (b) \text{ Galvano-sinusoidal.} \\ (c) \text{ Galvanic} \\ (d) \text{ Faradic} \\ (e) \text{ Sinusoidal} \end{array} \right\} \begin{array}{l} \text{in combination} \\ \text{with diathermic} \\ \text{current.} \end{array}$
5. High frequency $\left\{ \begin{array}{l} (a) \text{ from d'Arsonval generator.} \\ (b) \text{ from diathermic current generators.} \end{array} \right.$
(long wave) (short wave)
6. Currents derived from static electrical machines.

Galvanic Current

Galvanism or "Iontophoresis Without Therapeutic Ions." Galvanism has been practised since the middle of last century and employed for relieving pain and aiding resolution of inflammation after injury, but it is not known how it brings about the therapeutic results, which it undoubtedly does, in some pathological conditions. The principal diseases and morbid conditions suitable for treatment by galvanism are as follows:—

In *inflammation due to trauma*, the current aids in the absorption of inflammatory products. It thus usually causes rapid relief of pain and swelling in bruises and sprains, also in fractures and dislocations (after reduction). If the cause of the inflammation has not disappeared or ceased to act before the current is used, the relief, if any, is transitory.

In *osteoarthritis*, the galvanic current occasionally procures temporary relief, the best results being obtained in cases following trauma. In infective arthritis, it may assist in restoring mobility to the joints after the primary focal infection has been removed. In all cases of arthritis, except perhaps those which

are definitely due to traumatism alone, there is some fundamental cause or some focus of infection. Until these conditions have been effectually attended to, the results of any local treatment are usually disappointing.

The current is often effective as a pain-killer in cases of *neuritis and fibrositis* after medicinal and other treatment has failed. In cases of *indolent ulcer and indolent wounds*, the process of repair is stimulated or accelerated by the galvanic current (making the kathode the active electrode).

Erythema pernio (chilblain) responds rapidly to this current (the surging sinusoidal or surging faradic currents are equally effective, and if supplemented by local and general diathermy and appropriate internal medication, the effect is more lasting).

The endurance of voluntary muscular effort is very greatly increased by the passage of a galvanic current, which also mitigates or abolishes the fatigue felt during or after the effort. It has been suggested (Cumberbatch) that this "*refreshing action*" of the current is due to the migration of sarcolactic acid ions from the muscle fibres into the blood vessels or lymphatics.

The refreshing action possibly accounts for the beneficial results of galvanism in the treatment of *Raynaud's disease*. The frequency and severity of the attacks can usually be diminished by passing the current between the hands (or feet) immersed in water (anode) and the cervical (or lumbar) enlargement of the spinal cord (kathode).

It is possible that the relief of mental fatigue (psychasthenia) after the passage of the current through the head (cerebral galvanism), and the feeling of well-being after the current has been passed through the spinal cord, in cases of neuro-muscular neurasthenia, may be due to the removal of fatigue products by a process analogous to that which has been suggested in the case of tired muscles.

Some cases of *progressive muscular atrophy* are benefited by the application of the galvanic current to the spine. The same treatment often relieves the crises, pains, and unsteadiness of gait in *locomotor ataxy*.

Method of Treatment. The electrodes, except when used for special regions such as the middle ear, rectum, colon, uterus, etc., are metal plates made of thin sheet lead or tin alloy padded with several layers of lint or bath-towel material soaked in 1 to 2% solution of common salt at a temperature of about 100°F.

The padded electrodes are applied, one to the part requiring treatment (this is called the *active* electrode); the other (*indifferent* or *inactive* electrode) is applied to a distant part of the body away from the part requiring treatment. Before applying the padded electrodes, the skin to which they are to be applied should be washed with soap and warm water, and then well rinsed with warm water so as to remove the soap. Any cut, scratch, pimple, etc., however small, should be covered by thin sheet rubber, adhesive plaster or a drop of collodion.

The pads may consist of 16 layers of lint or 8 layers of bath-towel material of medium thickness, so that after they have been well soaked in the solution of salt (which constitutes the electrolyte) and wrung out they are not less than $\frac{1}{2}$ inch in thickness. During the passage of the current, the chemical caustics will be formed where the metal plate makes contact with the electrolyte, but if the pads are sufficiently thick, the metal plates are at a sufficient distance from the skin to prevent the chemical caustic reaching the epidermis and causing so-called "burns." The sodium ions at the positive pole and the chlorine ions at the negative will conduct the current between the metal plates and the skin, and thus complete the circuit, and the galvanic current will pass through the body.

When the padded electrodes have been accurately applied and bandaged on and the circuit is complete, the current is started from zero and very gradually increased, several minutes being expended before reaching the desired maximum. (During the application, the sensations felt at the skin surfaces should be *evenly diffused*. If the patient complains of a *point* of special pain, or if the milliamperemeter needle is observed to move forwards after a period of rest, apart from any movement of the current regulator, it may signify a breaking down of the skin resistance, and possible injury to the epidermis. Whenever these signs are noticed, the current should be reduced to zero, the pads removed and the skin examined. If the skin has been damaged, a dark grey area will be seen in place of the normal erythema.) It is allowed to flow at this strength for the appropriate time and then it is very gradually reduced to zero.

Current density, or the density of the current in the skin, is the number of milliamperes passing per square inch or square centimetre of electrode in contact with the skin.

Patients can rarely tolerate more than one or two milliamperes to the square inch to begin with, and during the first treatment it is not wise to exceed this amount. Patients have idiosyncrasies to electricity as well as to drugs. In different subjects the sensitiveness of the skin, even if the latter is apparently normal, varies exceedingly. Some subjects are liable to develop urticaria with a current density that produces no harmful effects in others. If, however, the skin develops urticaria, the current should not be passed again until the eruption has disappeared.

"If a part of the central nervous system (brain or spinal cord) or an organ of special sense (such as the eye, internal ear or semicircular canals) lies on the path of the current, the latter cannot attain as high a density as the skin without causing disagreeable sensation on the part of the patient."—Cumberbatch.

A *visible physiological change* is a *patch of erythema* which can be observed in the skin where it was covered by the electrodes. These patches of erythema are due to vasodilatation, not merely in the skin but according to certain evidence, in the deeper parts as well. The production of this "galvanic erythema" is the probable explanation of the beneficial results of galvanism when applied to a region which is the seat of passive congestion or of inflammation which has failed to resolve.

Kataphoresis or "**electric osmosis**" is the passage of water from the anode to the kathode during the flow of the current. Whether kataphoresis takes place in such a complex conductor as the body to any appreciable therapeutic extent is uncertain, although some authorities ascribe the good results obtained by "chlorine ionisation" of an ulcer, as being due to kataphoresis and not to the chlorine ions.

The phenomenon named **anelectrotonus** (the lowering or even temporary abolition of excitability and conductivity in

muscle or nerve at the anode) is sometimes cited as an explanation of relief of pain which can be procured by means of the current.

Iontophoresis with Introduction of Therapeutic Ions or "Ionisation," "Medical Ionisation," "Kataphoresis."

This is a form of treatment in which the galvanic current is used for the purpose of making ions possessing therapeutic properties migrate into the body.

The term *ionisation* as applied to liquids, strictly means or implies the disassociation of a substance into ions without the application of any external force, as is the case when a salt is dissolved in water. Inasmuch as the galvanic current in the body consists of streams of migrating ions, the term "*iontophoresis*" (signifying the *conveyance of ions*) is more applicable. The term *kataphoresis* as applied to this method of treatment is impressive but misleading.

The publication in 1900 by Professor Stephane Leduc of the first of a series of papers on the subject, may be said to mark the dawn of the ionisation era. In these papers, Leduc explained the principles of the subject, and indicated the proper technique and the directions along which success might be pursued.

Dr. Turrell, in summarising the *arguments against the theory of ionic medication*, says:—

- (1) That the function of the solution of salts or "drugs" with which the pads are moistened is to supply the ions necessary for the transmission of the current through mucous membranes or the superficial layers of the skin, an area which is normally very deficient in ions.
- (2) That the current is conveyed through the body by the tissue ions, chiefly, in view of their relative fastness, by the hydrogen kations and the hydroxyl anions.
- (3) That while a salt is in an ionic state, its chemical affinity is temporarily inhibited by its electrical charge, therefore as an ion it can have no therapeutic action.
- (4) That the very low velocity of the ions employed in medical treatments of this character, and the very low potential at which they are applied, entirely preclude the deep penetration claimed for them during the brief period allotted to an electric treatment.
- (5) That, according to Sir Oliver Lodge, "At a change of liquid another set of atoms continues the convection and nothing very particular need be noticed at the junction."
- (6) That such drugs as sodium salicylate, commonly employed, need to be present in the tissues in considerable quantities in order to exercise their therapeutic effect, and it is difficult to conceive that such drugs in the infinitesimal quantities which could possibly be introduced by ionic medication, could have any beneficial action.
- (7) That many workers of extensive experience have failed to detect any difference in these results provided the current is administered at a similar intensity and for a similar length of time, whatever salts or drugs are employed to moisten the pads.
- (8) That very careful experiments conducted both in this country and in France by experienced electro-therapists in collaboration with skilled chemists, have failed to detect the presence of the drug, after its attempted introduction by electrical means, deeper than the superficial layers of the skin.

The late Dr. Lewis Jones, who introduced ionic therapy into England, wrote as follows in the preface to his monograph entitled *Ionic Medication*:—

"The evidence which has thus (by a record of actual clinical results) been brought together, offers the best of proof that the practice of *ionic medication is a real addition to our means of healing disease*. In view of the facts adduced, we need not too greatly concern ourselves with academic discussions as to whether there is any effective penetration of ions or not. At least we may say that the procedures described have been followed by results of value.

"At the same time we may learn from this collection of cases that the best results have been gained in superficial affections, and we must not be too confident of securing brilliant effects in the more deep-seated affections."

Cumberbatch (*Essentials of Medical Electricity*) says: "In some diseases the therapeutic effects are certainly due to the ions that are introduced, although the current, apart from the ions that have gained entry, may assist in the production of these effects. In other diseases it is uncertain whether the special ions that are introduced play any part. In these, the therapeutic effects are to be attributed mainly, and some writers would say entirely, to the action of the current itself—that is, to the migration of the tissue ions."

As time has gone on it has been shown that fewer and fewer of the successful results in various affections are really due to the therapeutic ions that were introduced. In the past, a great variety of therapeutic ions have been employed. Nowadays the most commonly used ions are those of zinc and copper. Mercury magnesium, salicylic and iodine ions are occasionally employed. *Many authorities would restrict the list to zinc and copper ions.*

The lithium, iodide and salicylate ions have been made to pass through the skin (if applied at the proper poles) and have been detected in the urine and saliva. Leduc, experimenting upon rabbits with shaved sides, has not only proved the penetration of cyanogen and strychnine ions, but has shown that the former (CN) only enters from the kathode and the latter from the anode. In both cases the animals died. The entrance of these poisonous substances is not by diffusion, because control animals with pads soaked in solutions of potassium cyanide or strychnine hydrochloride in contact with the shaven skin, and kept there indefinitely, were not affected if the current was not made to flow. Furthermore, they were not affected if the current was made to flow and the pad electrodes were connected to the wrong poles of the source of current. Again, if a pad soaked in a 1 or 2% solution of zinc sulphate is applied to the granulations at the base and edges of an ulcer and kept there for 10 minutes, it produces no result that is visible to the eye. If, however, a metallic electrode connected to the positive pole of a source of galvanic current be placed over the pad (the indifferent electrode being applied elsewhere) and the latter allowed to flow for 10 minutes, the granulation tissue acquires a pearly-white colour not only on the surface but in the deeper parts. This white colour is due to the formation of a compound of zinc and albumen. The zinc ions have entered, and many have combined with the tissue proteins.

The *regions which can be treated by introduction of therapeutic ions* are as follows:—any region of the skin when inflamed, with or without ulceration, also fistulae and artificial sinuses which open on to it; any mucous membranes which are within reach of the active electrode, viz., those which line the following regions: nasal cavities, frontal sinus, maxillary antrum, eyelids, cornea, sclerotic, tongue, mouth, Eustachian tubes, middle ear (if the membrana tympani is perforated), anal canal, rectum, colon (as far as the splenic flexure), vagina, canal of the cervix and body of the uterus.

The electrodes when applied to the surface of the body, consist of metal plates and absorbent pads like those described in the treatment termed galvanism or "iontophoresis without the introduction of therapeutic ions." The "active" pads are soaked in the solutions containing the ions to be introduced. Electrodes of this kind may be applied to ulcers of the skin if not too small, the metal plates and pads being cut to suitable sizes. If the ulcer is only $\frac{1}{4}$ inch or less in width, a small pad of cotton wool soaked in the solution is placed over it, and then a zinc rod is applied to the pad. For the treatment of still smaller ulcers, cotton wool wrapped round the end of a zinc rod and soaked in the solution may be used. In the treatment of an intractable form of corneal ulcer known as Mooren's ulcer, a bare zinc rod is used. Bare rods of zinc or copper are also used in the treatment of narrow channels such as the cervix uteri and artificial sinuses of small calibre. (When the current flows, the metal, if connected to the positive pole, slowly passes into solution, so that ions are formed. These ions then migrate into the tissues.) In the treatment of internal parts such as the rectum, colon, maxillary antrum, middle ear, etc., the cavity is first filled with the solution, which is then connected to the source of galvanic current by a special metallic electrode.

The Council on Pharmacy and Chemistry and the Council on Physical Therapy of the A.M.A. have decided that ion transfer as a method of administering drugs be recognised by the Council with the understanding that the application of the method must at present be reserved to those especially trained and that the following precautions should be observed:

(1) If the sensation of the patient's skin is not normal on test, the current strength must be proportionately selected and carefully controlled according to the size of the electrodes and the ma. reading. (2) Electrodes must not be applied over denuded areas and must be very carefully applied over recent scar tissue. (3) Electrodes must be sufficiently large to avoid excessive current density. (4) The metal plate of the electrode must be evenly covered by the padding, leaving no bare edges in contact with the skin. (5) The pad covering the electrode must be evenly saturated with the appropriate solution. (6) The electrodes must be applied in good contact by means of even pressure. (7) The conducting wires must be fastened securely to the electrodes, so that the metal of the wire tip does not come in contact with the skin. (8) The current must be increased slowly at the start; while the milliammeter registers flow of current, the switch must never be opened or closed sharply. (9) The patient must be instructed to report at once any excessive pain or burning. No more current than the patient states is comfortable should be applied. A safe milliammeter reading should never be exceeded. During treatment the milliammeter reading usually increases as skin resistance decreases; a decrease usually indicates that the pads have become partially dry. In remoistening of the pads the current must be turned off, after which the electrodes are removed and the pads moistened evenly and reapplied. (10) The strength of the current must be decreased if the patient complains of annoying sensations. If this does not provide relief the current must be turned off and the electrodes removed.

—*J. Amer. med. Ass.*, ii/1941, 360.

Properties and Sources of Various Ions. The old mnemonic used was Metals and Alkaloids are Positive (M.A.P.). Of recent years the more academic mnemonic, Hydrogen, Alkaloids and Metals are Positive (H.A.M.P.) has been adopted. All other ions are negative.

When they are prepared for use, the strength of the solutions should as a rule be made 1%.

If *iodine* is dissolved in potassium iodide the solution for use should contain 0.5% of each. The strength of *adrenaline* should be 1 in 5000.

The ions of *zinc*, *copper*, *silver* and *mercury* all possess germicidal and cauterising properties. They do not penetrate deeply owing to the readiness with which they enter into combination with the tissue ions and form more or less insoluble salts. When these specific ions pass into the cells they destroy the protoplasm

and enter into combination with the proteins formed by its decomposition. In this way, there are formed the insoluble albuminates of these four metals. It is probable that zinc ions penetrate the more deeply, therefore they are used in preference to the others in the treatment of ulcers and sinuses.

Ions with Positive Charges.

Zinc ions are most conveniently derived from a solution of zinc sulphate. They are employed in the treatment of ulcers, sinuses and infected conditions of mucous membranes. The zinc albuminate is an ideal dressing, being tenacious and sterile. It remains in position for 7 to 10 days. During this time the underlying tissues cannot be re-infected from without. Within the layer, bacteria are killed and capillaries are sealed.

Copper ions (derived from a solution of copper sulphate) possess the same therapeutic properties as those of zinc.

Mercury ions (derived from a solution of mercuric chloride) possess the same properties as zinc, copper and silver ions. They are antiseptic, and may be used for the treatment of syphilitic ulcers.

Silver ions (derived from a solution of silver nitrate) are not frequently employed. They form very insoluble salts when they meet tissue ions, including the chlorine ions, and therefore the ions of this metal migrate for a much shorter distance than those of zinc and copper.

Magnesium ions (derived from a solution of magnesium sulphate) do not possess cauterising power. They are used in the treatment of small multiple flat warts which occasionally occur on the hands or face. If this method is going to be successful, only one or two applications are necessary.

Lithium ions (best derived from a solution of lithium chloride) have been used for the treatment of gouty arthritis. The passage of the galvanic current without the lithium produces equally good results, especially when combined with salicylic ions.

Quinine ions (derived from a solution of quinine hydrochloride) have been used in the treatment of neuralgia and painful neuritis. Although they are credited with analgesic properties, they are apt to cause marked skin irritation (e.g., when applied to the face in cases of trigeminal neuralgia) so that sufficiently massive currents cannot be comfortably borne.

Cocaine ions (derived from a 10% solution of cocaine hydrochloride) produce superficial anæsthesia. These are usually combined with *adrenaline ions*, in order to procure vaso-constriction at the same time as local anæsthesia. The results are unreliable—a local injection of procaine and adrenaline is preferable.

Histamine ions (derived from a solution of histamine hydrochloride) have been recently claimed to be efficacious in the treatment of non-infective affections of the muscles and joints. Katexon

Foil is used. This consists of sheets of filter paper saturated with a solution of histamine hydrochloride and covered with a thin coating of aluminium which acts as a conductor of the current. Before use, the Katexon Foil must be soaked in water.

Histamine ointment gives better results and is more convenient in use than solution, the part affected being smeared with the ointment and a lint pad, wrung out of saline, placed over the part and connected to the anode of the apparatus. Patient given the maximum milliamperage which can be tolerated, frequently up to 100 or 150 ma., the current being raised to maximum as rapidly as compatible with patient's comfort and maintained as high as patient will allow to end of sitting. The tolerance of a patient both as to time and milliamperage varies greatly from day to day. There is immediate tingling and intense itching at site of anodal pad, with feeling of local warmth, and patient must be told how to distinguish between this and burning due to concentration of current; also to complain if limb begins to throb, face flushes or if there is pain in the head. To avoid bad headache, current must be cut off at first suspicion of flushing, and reduced if throbbing or excessive heat occurs. The relief of pain, either complete or partial, may last a few hours, a few days or permanently. Undesirable results, indicating immediate cessation of sitting, include headache, tachycardia, constriction of chest, with breathlessness, burning, and faintness.

Treatment may be given daily. Has no place in the treatment of infective arthritis, acute rheumatoid arthritis or where there is marked failure of compensation in the heart, but of special value in fibrositis and neuritis, which it is possible to cure. Massage with the ointment also satisfactory but less so than ionisation.—F. S. Mackenna, *Lancet*, i/1934, 1223.

Many patients who have taken a course of baths for several years are emphatic in their statements that histamine has greatly increased the benefit received; undoubtedly it also increases the rate of recovery. It may be said to fail in cases of the rheumatoid type, where the joints are swollen and "doughy" and the skin clammy, but in all others, and particularly in villous arthritis, fibrositis, and neuritis, its value is unquestionable. An analysis of 100 cases.—F. S. Mackenna, *Lancet*, i/1936, 365.

Ions with Negative Charges.

Acetyl- β -methylcholine ions (derived from a solution of acetyl- β -methylcholine chloride). The application of aqueous solutions of this drug by iontophoresis is the best means to obtain the local effects of the drug on the extremities. Systemic effects are produced by this method but are less pronounced than when the drug is given orally or by injection.

For administration of the drug by iontophoresis it is customary to use a 0.2 to 0.5% solution in distilled water. The initial treatment should not exceed 5 to 10 ma. for 30 minutes. Subsequent treatments usually require from 25 to 30 ma. for 20 to 30 minutes, with an interval of 3 or 4 days between treatments, the number of treatments necessary varying with the patient and the type of lesion. In Raynaud's disease and scleroderma ten or more treatments may be necessary to secure improvement; in chronic rheumatoid arthritis the treatments may be reduced to intervals of a week after the first four to six treatments. In varicose, indolent, and gangrenous ulcers treatment may be given daily at the start to promote granulation of tissue and then reduced after the first few treatments to two or three times a week. During and after treatment the patient should be covered and protected from draughts and should be kept quiet and warm for about 30 minutes. It should only be used by those specially trained and should not be used directly over ulcers or open wounds and only with care over scar tissue. Idiosyncrasy to the drug may result in difficulty in breathing. If this is noted the treatment should be stopped and the patient raised to a sitting position. If the symptoms do not subside atropine sulphate should be given hypodermically at once.—Report of the Council on Pharmacy and Chemistry, *J. Amer. med. Ass.*, ii/1941, 861.

Adrenaline ions (derived from a solution of adrenaline dihydrogenphosphate). Some of the disadvantages of adrenaline injections in the treatment of asthma, e.g., short duration of action and

unpleasant reactions, may be overcome by adrenaline iontophoresis. A solution of adrenaline dihydrogen phosphate with pH of 4 to 5 is employed.

Equal quantities of adrenaline base and phosphoric acid are added together so that the final concentration of adrenaline is about 1%, the pH being adjusted colorimetrically. The area of application in the arm or leg is preferably about 50 sq. cm. The adrenaline solution is placed on cotton, and each area is given a current of 5 to 7 ma. for 10 to 15 minutes with usually three areas being covered successively in a single treatment. (By using larger areas and increasing the current and time it is conceivable that larger doses may be given.) Constitutional reactions, such as tremor, palpitation or headache, have been less than those following a subcutaneous injection of 5 m. of the 1 in 1000 solution of adrenaline hydrochloride.—Abramson, per *Brit. med. J.*, ii/1940, 293.

Chlorine ions (derived from a solution of common salt) are used for softening superficial scars which are the result of trauma or burns, and for loosening superficial adhesions. The best results are produced by a combination of radiant heat or diathermy, "chlorine ionisation," and massage and movements in direct sequence and on the same day. If the scar be painful, salicylic ions are preferable to chlorine ions. The modern belief is that chlorine ions have no direct "sclerolytic" action and that the beneficial results are probably due to kataphoresis.

Iodine ions (derived from a solution of potassium iodide) have also been used for softening scar tissue, but it is doubtful whether they exert any specific action in the process. They have antiseptic properties and may therefore be introduced into syphilitic ulcers. The addition of free iodine to the solution of potassium iodide increases their germicidal action, and makes them very successful in the treatment of chronic non-specific infected ulcers and sinuses.

Salicylic ions (derived from a solution of sodium salicylate) possess weak germicidal powers and are well borne by the tissues.

They have been found very efficacious in relieving both superficial and deep conditions when attended with pain, e.g., painful scar tissue and ulcers, and trigeminal neuralgia.

INTRANASAL IONISATION IN HAY FEVER AND ALLIED CONDITIONS. The treatment consists in thoroughly anæsthetising the entire nasal cavity; following this the nasal chambers are again packed with cotton strips saturated with a metallic solution. This consists of 1% zinc, tin, and cadmium chloride in glycerin. A bare copper wire is placed in the packing avoiding direct contact with the nasal mucosa. This acts as the anode and both sides of the nose are treated simultaneously. The cathode consists of a pad soaked in saline solution and brought in contact with the patient's palm. The galvanic current is gradually turned on until the ammeter registers about 10 milliamperes and the treatment is continued for about fifteen minutes. It is important to bear in mind that excessive current and prolonged time period may result in coagulation of the tissues. When the packs are removed the nasal mucosa is covered with a greyish film. The reaction begins within a few hours following the treatment. The turbinates and other parts of the nasal mucosa become swollen and breathing becomes impaired. The patient complains of nasal obstruction, of varying amounts of pain about the nose and face with headache. On the second day the pain subsides; the nose, however, is still obstructed. At this time a gelatinous membrane forms within the nose. This the patient is able to expel on the third day. By the end of the third or fourth day the patient is relieved of his symptoms. At the end of six days rhinoscopy reveals a comparatively normal appearance of the nose.—S. W. Garfin and S. M. Pearl, *New Engl. J. Med.*, 1936, 214, 245. See also P. Franklin, *Brit. med. J.*, i/1932, 751.

As a result of treatment by intranasal zinc ionisation of 243 cases of hay fever at St. George's Hospital during 1936, the ages of the patients ranging from 5 to 77 years, complete relief of all symptoms was obtained in 57.6% and considerable relief in 36%. The technique is safe and complications are not met with. The routine practice was to give three treatments at intervals of one week.—L. D. Bailey and Clive Shields, *Brit. med. J.*, i/1937, 808; P. Franklin, *ibid.*, i/1939, 871. See also L. D. Bailey and Clive Shields, *Brit. J. phys. Med.*, July 1936, 52; Frank Cope, *ibid.*, Aug. 1936, 76; A. R. Hollender, *ibid.*, Sept. 1936, 96.

Diseases and Morbid Conditions for which "ionisation" is a valuable form of treatment often after other measures have proved unsuccessful.

CHRONIC INFECTIVE PROCTITIS. Zinc ionisation generally successful—using a special electrode.

ULCERATIVE COLITIS. J. Curtis Webb (*Lancet*, ii/1905, 1361) advocated the use of silver ions, using a special electrode and technique. Zinc ions were afterwards used instead of silver ions and Julius Burnford (*Brit. med. J.*, ii/1930, 640) described the results of the treatment in 28 cases.

MUCOUS COLITIS. Salicylic ions may be used (pelvic diathermy applied by the rectal route is often successful).

CERVICITIS. Very successful. Begin treatment by placing a zinc electrode in the canal of the cervix, make it the *kathode*. This causes a migration of hydroxyl ions into the cervix, where they destroy organisms and convert the cells into alkali-albumen. There is also an increase of the secretion from the cervical glands, thus establishing efficient drainage. It is of special value when the discharge is slow, thick and adherent. When the discharge is thinner and more copious, the active electrode should be connected to the positive pole of the source of current; zinc ions are thus introduced and zinc albuminate is formed. (For full details consult E. P. Cumberbatch's *Essentials of Medical Electricity*.)

CHRONIC SUPPURATIVE OTITIS MEDIA (if there is an opening in the membrana tympani). If there is no caries of the bone, no papilloma or cholesteatoma, two or three treatments by zinc ionisation, using 0.5% solution of zinc sulphate, are usually effective. The solution is introduced by way of a special electrode as recommended by Friel ("Zinc Ionisation and Zinc Electrolysis in Treatment of Chronic Otorrhea," *Trans. Roy. Soc. Med., Otol. Sect.*, 1921), or by a special electrode designed by W. Claughton Douglass ("An improved method for the treatment of otitis media by ionisation," *Brit. J. Bio-phys.*, 1930). A current of 2 to 3 milliamperes is passed for 10 minutes.

MAXILLARY ANTRUM AND FRONTAL SINUS. Chronic inflammation of the mucous membrane of these parts can occasionally be brought to an end by zinc ionisation. Sometimes salicylic or iodine ions are introduced. Special electrodes and technique.

CORNEAL ULCERS. Use retractor, cocaineise eye, instil fluorescein. A tuft of cotton wool soaked in a 1% solution of zinc sulphate is wound round the end of a zinc rod. The free end of the tuft is placed on the ulcer, the rod is connected to the conducting wire leading to the positive pole of the battery. A current of 1 to 1½ milliamperes should be passed for 1 or 2 minutes. If the ulcer does not heal after 10 days a second application should be made. One application is sufficient to effect a cure if the ulcer is small. In treating "Mooren's ulcer," after cocaineising and instilling fluorescein, apply a zinc rod, $\frac{1}{16}$ in. diameter with its free end rounded, to the edge of the ulcer. A current of half a milliampere is passed, and the rod is moved very slowly along the edge. Each part covered by the zinc rod should receive the current for 15 seconds. After the lapse of 14 days, instil fluorescein and treat again any part which stains.

NON-SPECIFIC ULCERS OF THE SKIN. Zinc ions are generally introduced. The resulting pearly-white zinc albuminate is a tenacious, sterile, protective covering, and until it disappears no further electrical treatment should be given. One treatment is often sufficient. Zinc ions are most useful in cases of infected ulcers; sometimes "varicose" ulcers can be healed by the same method. If the ulcer be inflamed or painful, one or two preliminary treatments by salicylic ions are often effective. Iodine and salicylic ions sometimes succeed when zinc ions fail. When an ulcer is callous, and infection is not the evident cause of its persistence, chlorine ions should be introduced.

SYPHILITIC ULCERS should be treated by the introduction of mercury or iodine ions.

PERFORATING ULCERS may be treated by introducing the negative ions present in a 1% solution of iodine in a solution of potassium iodide of the same strength.

ARTIFICIAL SINUSES. Success can only be expected when the sinus is more or less straight and has no side channels, does not lead to carious bone and does not contain any foreign body (pledgets of paper or cloth are transparent to X-rays). First cleanse the sinus to prevent any part of its surface from being shielded by pus or debris. It should then be well filled and even distended with the solution, and the electrode in the form of a zinc or copper rod should be so contrived as to be equally close to all parts of the area. At the commencement of the treatment, the more distant part of the sinus should receive most attention, so that it may heal from the bottom.

If zinc ions are used, a zinc rod is made the *anode*; if iodine ions are used, a zinc rod is made the *kathode*. If the sinus is not too narrow, a zinc rod carefully covered with cotton wool soaked in the solution is passed along the channel. If it is too narrow, a bare zinc rod is used.

The Galvanic Current Used for the Removal of Foreign Ions.

C. H. C. Dalton (*Brit. med. J.*, ii/1929, 297) reports a case of footdrop due to arsenical poisoning, which was successfully treated by the galvanic current. The affected muscles were stimulated by interrupted galvanism thrice a week. The affected limb was then placed in a kathodal foot-bath and, in addition, a pad was placed, reaching up the skin of the leg. The unaffected leg was placed in an anodal leg-bath. After 14 days, the skin under the pad was noticed to be scaly. The scales and the fluid wrung from the pad both showed the presence of arsenic by Marsh's test. The patient was first treated on April 10, 1928—on May 30 the condition almost approached the normal. When examined on June 20 the affected muscles had completely recovered, and normal sensation of the skin on the front of the leg was found. The arsenic in such cases is probably in the form of hydrochloride.

A somewhat similar technique is employed in the treatment of workpeople employed in lead factories—the active electrode is kathodal. After each application, lead has been found in the bath water, even when the patient has been out of work for 10 weeks, and was, therefore, unlikely to have any lead dust present on the surface of the skin. A distinct relief of the symptoms of lead-poisoning was observed, in addition to an improvement in the general health of the patients submitted to the treatment, which is not only preventive but curative as well. (Sir Thomas Oliver: *Lead Poisoning from Industrial, Social and Medical Point of View*, 1915.)

When the galvanic current traverses a fluid containing micro-organisms in suspension, the latter migrate towards the anode. It is likely that this movement takes place where the current is directed through infected fluids, and if the organisms can reach the anode they will be destroyed. A method based on this principle is used by Dr. Charles Russ for clearing the urethra of gonococci.

Electro-chemical Cauterisation, Surgical Ionisation or Electrolysis is a form of treatment in which abnormal or unnecessary tissue is destroyed by chemical caustics produced within it by means of the galvanic current. Caustics such as sodium hydroxide and hydroxyl ions or hydrochloric acid and hydrogen ions are derived from the sodium and chlorine ions in the tissue fluids. On the other hand, when zinc ions are to be used for cauterising purposes, they are derived usually from the metal itself, which is placed in contact with the tissue to be destroyed. When the metal, in the form of a zinc needle, is connected to the positive pole of a galvanic battery and the current is made to flow, the metal passes into solution, so that its ions are formed and they migrate into the tissue where zinc albuminate is formed. If the positive electrode is made of platinum or platinum-iridium, the tissue in its vicinity is transformed into acid albumen. At the kathode, whatever metal the electrode is made of, hydroxyl ions and sodium hydroxide are formed, and the tissue in the vicinity of the needle is transformed into alkali-albumen.

The details of this method of treatment differ according to the tissue which has to be treated. The active electrodes are in the form of needles, varying in width from 0.2 mm. to 1 or 2 mm. Their ends are rounded if they are to be introduced into channels, or pointed if they have to be inserted into tissue. The finer electrodes are best made of an alloy of platinum and iridium. Thicker electrodes are made of hardened zinc. The electrodes are attached to suitable holders made of ebonite or fibre, except in the case of multiple-needle monopolar electrodes, in which each needle is soldered to the bare end of a short length of insulated wire. The other end of each wire is soldered to a common terminal and this terminal is connected to *one* pole of the battery. They may be monopolar, multiple-needle monopolar, bipolar or multiple-needle bipolar.

A monopolar (or unipolar) electrode may consist of one, two or more needles. When it is used, the circuit must be completed by means of an indifferent electrode—a padded metal plate like that already described. When the electrode is bipolar or multiple-needle bipolar, all the electrodes are active.

The *advantage of cauterising tissue by the electro-chemical method* is the fact that the method is under exact control of the operator, so that the current can produce the amount of caustic desired precisely in the situation desired with little subsequent reaction and the formation of soft non-contracting scars. The limitation of the method is the fact that only a small amount of tissue can be destroyed at each application or insertion of the electrode. More tissue can be destroyed if inserted successively into adjoining regions, but this process is slow and tedious both to the patient and to the operator. If the electrode consists of a group of needles, the tissue can be cauterised at a quicker rate.

Therapeutic Field. Electro-chemical cauterisation is an efficient form of treatment for certain maladies and disfigurements of the skin and mucous membranes, such as hypertrichosis, cavernous nævi, stellate veins, telangiectases, newly grown venules in the skin, nævi composed of separated vessels and pedunculated warts. It is also efficient in the treatment of cases of chronic peripheral neuritis, such as sciatica and brachial neuritis.

HYPERTRICHOSIS (superfluous hairs). The procedure requires much practice, skill and patience. Thick and dark hairs, not too close together, are easiest to remove. (Fine, downy hair should not be thus treated.) When a large surface has to be done, it is better to remove hairs from every part and not from one spot, otherwise a troublesome ulcer would probably result. About twenty is the largest number that can be removed at a single sitting.

CAVERNOUS NÆVI. Use a Lewis Jones' multiple-needle bipolar electrode. Sterilise needles in the flame of a spirit lamp, and then push them into the nævus at the level of the skin. After current has flowed for half a minute, partially withdraw the needles and push them in again in a different direction. Repeat process if necessary, so that the needles explore all parts of the nævus, and pass through its blood vessels. Current should not exceed 20 milliamperes for each inch of positive needle inserted, otherwise a slough is apt to be formed. Give special attention to the margin, and supplement if necessary with the galvano-thermo-cautery. At interval of 4 weeks repeat treatment if necessary.

STELLATE VEINS. (Spider nævi). Insert zinc needle (anode) about $\frac{1}{4}$ in. into central vein ("the body of the spider") from which the others radiate. Current of 1 milliampere for one minute.

TELANGIECTASES AND NÆVI, COMPOSED OF VESSELS THAT ARE SEPARATED FROM EACH OTHER BY INTACT SKIN. Insert zinc needle (anode) to depth of about

$\frac{1}{8}$ in. Current of 0.5 to 1 milliampere (according to size of vessels) for 30 seconds. Transfix each vessel at intervals.

"PORT-WINE STAINS" may be treated by zinc needling. Electrode is inserted successively in closely adjacent regions. Current of 1 or 2 milliamperes for 30 seconds. Process so very slow that except for small naevi, electro-desiccation or radium is preferable.

WARTS, PEDUNCULATED, SESSILE OR SEMI-SESSILE, and "senile" warts and soft papillomata of the skin. Transfix base with zinc needle (anode). Current of 1 milliampere passed for 1 minute. If base of wart or papilloma is more than 2 or 3 mm. transfixion should be repeated in parallel lines. Flat warts are generally unsuitable for zinc needling—electro-desiccation is a better method.

MOLES. Non-pigmented hairy moles should be treated by epilation. If the mole does not disappear, use zinc needling in different directions. Electro-desiccation may be supplemented or substituted.

CHRONIC PERIPHERAL NEURITIS: NEURO-FIBROSITIS. TREATMENT BY GALVANIC ACU-PUNCTURE. Bare the region where patient feels the pain, moisten skin with warm saline. Pad electrode is applied to the skin of another part and connected to one of the terminals of a medical induction coil or a source of sinusoidal current. This is the indifferent electrode. (The writer always uses the secondary current from a Lewis Jones' coil.)

The active electrode is a small button electrode about $\frac{1}{16}$ in. in diameter, mounted on the end of a suitable holder. This is connected to the other terminal and is applied to the moistened skin. The current is started and increased until it produces a painless sensation of "pins and needles." The electrode is then moved over the region where the patient refers the pain. If the case is one for which galvanic acu-puncture is suitable, the passage of the electrode will detect a point or points where the current produces *acute* pain. These hypersensitive points are confirmed by repeated exploration from different angles. Slight shifting of the position of the button electrode causes instant disappearance of the acute pain, and the return of the painless sensation of "pins and needles." When confirmed, the "painful faradic points" are marked by a skin pencil with a ring or square round the electrode, and are then subjected to the following treatment:—Tincture of iodine having been applied, a few drops of procaine and adrenaline solution are injected just under the skin at the centre of the spot to be treated. The indifferent electrode is left in position, but is now connected to the *positive* pole of a source of *galvanic* current. The *negative* is connected to the active electrode. The active electrode is a steel needle which is attached to a suitable holder. The needle is insulated except for a distance at its free end (which distance varies according to the probable depth of insertion) by celluloid enamel.* Ordinary sewing or darning needles are quite suitable. The writer, in certain cases, uses a straight blade-shaped Hagedorn needle.

The point of the needle is inserted at the opening made by the syringe needle and should follow the same direction. A galvanic current of one or two milliamperes is passed and the needle electrode is inserted a little more deeply or its point moved about like a probe until the patient feels *acute* pain, often radiating. If the pain be very severe, keep the electrode in exactly the same position and at the same angle, reduce the current to zero and then inject a few minims of procaine and adrenaline solution down the side of the needle electrode as deeply as the point of the needle. After 2 minutes' interval, the current is again passed (still keeping the needle electrode in exactly the same position), and, owing to the second injection of the local anæsthetic, the patient can usually tolerate a current of 5 or 6 milliamperes for 5 minutes. The current is then reduced to zero, the needle is withdrawn and a collodion dressing applied. One, two or three hypersensitive spots, according to the sensitiveness of the patient, can be thus treated in succession at the same sitting. If a spot has been exactly located

*The insulating enamel is made by dissolving some black celluloid in a mixture of acetone and amyl acetate. The proportions are:—Acetone 47 parts; amyl acetate 47 parts; black celluloid 6 parts. The night before the treatment the needle is dipped into the enamel. The next day the enamel on the electrode will be dry. It can then be scraped away from the free end to the desired extent. The point, before insertion, is momentarily dipped into spirit. After two or three sessions of treatment, the electrode should be held for a moment in the flame of a spirit lamp so as to burn off the remains of the enamel. It can then be dipped in the solution again. The formula of the solution was devised by the late Dr. Muir of Exeter.

and correctly treated, there is no subsequent pain except perhaps a little "needle soreness," and a repetition of the exploration with the faradic or sinusoidal current 3 or 4 days afterwards, will fail to produce any pain when the testing electrode is moved over the spot which was treated. If the cause of the neuritic pain be still existent, some other tender "faradic points" may subsequently be found. They are treated in the same way. The hyperæsthetic faradic points are found so frequently in the same small areas, that an expert can usually know beforehand where he will find at least some of them.

If the patient be very nervous, it is advisable, when testing for the proper "pins and needles" strength of the faradic current, to move the active electrode over the corresponding region on the *opposite* side. The beneficial effects of this form of treatment are probably due to the cauterising action at the negative pole being exerted, not on a main nerve trunk, but rather on one of its sensory branches along which the pain is referred to the surface.

The galvanic current produces caustic chemicals below the skin in accurately located spots. Galvanic acu-puncture can be used in all cases in which some specialists use injections of absolute alcohol, and is, in the writer's experience, preferable and less painful. This form of treatment should be reserved for *chronic* cases.

The source of galvanic current for iontophoresis and electro-chemical cauterisation may be D.C. main, A.C. main by means of a rectifier, batteries of wet or dry Leclanché cells, accumulators, or dynamo and driving plant (private installation). Those sources should be used, for medical purposes, with the interposition of suitable resistances.

Galvanic Current Used for the Stimulation of Muscle or Nerve.

When treatment by "galvanism" or "iontophoresis" ("ionisation") is being administered, it is necessary to increase and decrease the current with adequate *slowness* in order to avoid causing pain or discomfort to the patient. If a current passes along the body with *constant* strength, it does not stimulate the muscles or *motor* nerves. The *sensory* nerve-endings are, however, stimulated, possibly in consequence of the accumulation of ions in the skin. This stimulation persists during and after the passage of the current, and it may be the cause of the vasodilatation that accompanies it—a feeling of warmth is experienced, and the skin under the electrode acquires an erythema. If the current be *suddenly* increased or *suddenly* decreased, it stimulates both the sensory and motor nerves. If the reduction is less sudden, the sensory nerves alone will be stimulated. If the reduction is very gradual, there will be no stimulation. The two factors which determine the stimulating power are the strength attained by the current and the time occupied by the current in rising to its maximum or in falling to zero. When used for the purpose of stimulation, the galvanic current must be made interrupted, surging or alternating.

Frequently Interrupted Galvanic Current is obtained when a metronome interrupter is placed between the battery of cells and one of the electrodes. This current is used for producing contraction of muscles which show the reaction of degeneration, the active electrode being placed, in turn, over the motor point of each of the affected muscles. When used for testing the reactions of muscle and nerve, the current is interrupted by means of a make-and-break key which is operated by hand.

Frequently Interrupted Galvanic Current (the Leduc Current). The Leduc interrupter is introduced into the current, which can by this means be interrupted at a much more rapid rate than when the metronome interrupter was used. Although the Leduc current can be used for the purpose of exercising muscles in cases of paralysis, it is practically only used for the treatment of *causalgia*—a very painful affliction which may follow nerve injury.

Surging Galvanic Current. This is a current which rises and falls very slowly between zero and maximum, one second or more being occupied by the rise and fall. The easiest method of obtaining such a current is to move rhythmically the regulating device attached to a "galvanostat" or "ionostat" (instruments connected to the main supply with lamp and water resistances). By moving the regulating handle the operator can make the current surge between zero and the desired maximum. By altering the direction of movement of the handle, the current can also be made to flow in either direction. The surging current is used in the treatment of paralysis in cases where one set of muscles shows the reaction of degeneration and the antagonists show reactions of normal type. The current causes contraction which is limited to muscles showing reaction of degeneration. The paralysed muscles therefore are not stretched. The affected arm or leg can be immersed in water and the current directed along the limb. If an interrupted galvanic current were passed along the immersed limb, the normally reacting muscles would contract with quick twitches and would momentarily stretch the paralysed muscles before they began their sluggish contraction.

Infrequently Alternating Galvanic Current. This current may be obtained from the galvanic current by introducing a *metronomic reverser* into the circuit. This metronome has four mercury cups, two on each side. When the swinging arm is on one side, the current flows in one direction to the patient, and when it is on the other side, the direction of flow is reversed. This alternate anodic and cathodic stimulation is useful in the treatment of muscles showing the reaction of degeneration. Some denervated muscles in a limb give a larger contraction in response to anodal stimulation, others may give a larger contraction in response to kathodal stimulation.

If the alternating galvanic current passes along the limb, all the denervated muscles will receive a more even degree of exercise than they do when the interrupted galvanic (unidirectional) current passes. In the application of the galvanic current, bath electrodes may be used—the water in the bath with the metal or carbon introduced into it constitute the electrode or electrodes. The most commonly used are the leg and arm Schnee baths. Baths are made of porcelain or china, or still better, owing to their lightness, of aluminium lined on the interior by waterproof insulating material. In Raynaud's disease, for instance, the arms or legs are immersed in these baths and connected to the positive

pole. The electrode over the spinal cord is made the kathode. In other cases, bath electrodes are convenient for the purpose of completing the circuit when a pad electrode is applied to the part requiring treatment.

Faradic Currents

The faradic current is obtained from an induction coil. The most important property of the faradic current is its power to stimulate the excitable tissues, particularly motor nerves. It is therefore able to produce contraction of voluntary muscle, and it is probable that, in consequence, many or all parts of the body are reflexly stimulated. The faradic current is chiefly used for the treatment of paralysis. Its therapeutic properties in this condition are not due entirely to its power to cause contraction of muscle. If the latter shows the reaction of degeneration, the faradic current is unable to cause it to contract, but it is nevertheless able to hasten recovery and to relieve some of the symptoms which may accompany paralysis, viz. cyanosis, chilblains, trophic sores and other results of defective circulation.

The faradic current is applied in the same way as the galvanic, and similar electrodes are used. Since it causes muscles to contract, it is necessary to introduce into the circuit some device which will rhythmically interrupt the current. The muscles will therefore relax during the period when the current is not flowing, and fatigue which would be caused by continuous contraction is prevented.

Apart from the prevention of fatigue, currents which are rhythmically interrupted produce better results than those which flow continuously. *This is an important general principle*, and it is true of *all* currents used for the purposes of stimulation, whether the muscles contract or not. Rhythmic interruption of the faradic current can be produced by sliding the secondary coil to and fro along the primary, till the sensation perceived by the patient is as strong as can be borne without discomfort. It is then moved off until no sensation is perceived. About two seconds should be spent in each movement. An alternative method is to include a metronome interrupter in the circuit, and regulate the rate of swing so that the current flows for about two seconds and is interrupted for the same period. Another method of procuring the necessary periods of rest between those of stimulation is the manual or labile method, to which reference has already been made. The active electrode is a padded metal disc, considerably smaller than the region requiring treatment.

This electrode is rhythmically stroked over the part and lifted at the end of each stroke. The pad of the electrode is soaked in warm saline—if the padded electrode is occasionally rubbed over a cake of soap the application is more pleasant. This *manual* or *labile* method is suitable for regions such as the face, foot, etc., where the accurate application of electrodes is less easy; it also constitutes a form of massage. Still another method is to make

the current *surge*, or wax and wane. After each surge there should be a period of rest before the next surge begins. The duration of each surge should be from one to two seconds, and that of each period of rest should be the same. Some of the types of surging devices most frequently used will be described when discussing *sinusoidal currents*.

As one of the most important uses of the faradic current is to procure contraction of muscle without painful sensation in its passage through the skin (except in some cases of hysteria, drunkenness, and malingering, which require *painful stimulation*), the choice of coil is important. The Lewis Jones sledge coil, the Tripiier coil (reintroduced of late years as the Physio coil), and the Spamer coil, are all suitable.

Therapeutic Uses of the Faradic Current. This current is used for practically the same purposes as the quick sinusoidal current. Faradic coils are portable, and are useful in cases where only occasional treatment is required. Where work is heavy and continuous, the sinusoidal current is more suitable.

In treating cases of paralysis or weakness of muscles, the general rule is to use the faradic current if the muscles will contract to it, but if not, to use the galvanic current. All forms of electrical stimulation are of use in the treatment of paralytic conditions, if the current be rhythmically interrupted or surged. The general treatment of paralysis will be described later on.

The faradic current may be usefully employed in the treatment of chilblains, constipation, fibrositis with adhesions (in these cases, use the Physio coil with an adjustable interrupter so adjusted as to produce strong muscular contractions about one per second), incontinence of urine (strong applications are necessary), and hysterical aphonia (strong applications are necessary). In the treatment of obesity by faradic current, the muscles are made to contract against resistance (heavy sandbags) and to do work.

The faradic current is also used for procuring strong painful sensation of sensory nerves. This treatment is used for hysteria; also for producing counter-irritation in neuralgia. In such cases, the current from a coil with a long secondary winding must be used, as a high voltage is required. A fine wire brush is used as the active electrode and is stroked over the skin.

Sinusoidal Alternating Currents

In these currents, the rise from zero to maximum and the fall from maximum to zero are gradual. Further, on reaching zero, there is no period of intermission but a second rise to maximum and fall to zero in the opposite direction. The currents are called *sinusoidal* because their graphic record resembles a sine curve.

Quick Sinusoidal Alternating Currents. These currents are devised from (a) A.C. dynamos; (b) D.C. dynamos; or (c) from accumulators. In utilising the current from either (b) or (c), a *rotary converter* is employed. Rotary converters are fitted to the various forms of so-called "Universal Apparatus," such as the Pantostat, Multostat, Polystat, Plurostat, etc., according to the makers. The expression "quick" refers to the duration of each period of flow in one direction before reversal of the current. When the duration is not less than 1/50th of a second, the sinusoidal current may be called "quick."

These alternating currents stimulate the excitable tissues, but they cannot cause ions to migrate—they merely cause them to move to and fro. They cannot therefore be used in treatment by "ionisation." They can, however, produce chemical changes at

metal electrodes, but in insufficient degree to harm the tissues.

The quick sinusoidal alternating current stimulates muscle and nerve. It causes contraction of muscles which show normal reactions, but it is unable to cause contraction of muscles which show the reaction of degeneration. Nevertheless it possesses therapeutic value in the treatment of paralysis even though it cannot make the muscles contract. Cyanosis and trophic lesions which accompany paralysis of large groups of muscles (as in cases of infantile palsy) diminish or disappear after treatment by this current. Furthermore, in such cases the return of some degree of power to muscles which have long remained powerless, either under expectant treatment or with massage, exercises, etc., is frequently noticed after the sinusoidal current has been utilised for a few weeks.

The quick sinusoidal alternating current (rhythmically varied) is of value in the treatment of *weakness and paralysis of muscles as a result of disease or injury*. In post-influenzal general muscular weakness and mental depression it is very beneficial. Goodall and Wallis found that though the excretion of creatinine is, in general, deficient in the insane, the sinusoidal bath tends to increase it, and that treatment with warm baths alone had very little influence on the creatinine excretion (*J. ment. Sci.*, April 1910).

The quick sinusoidal current is of considerable value in the treatment of *chronic cases of neuritis*. In acute or sub-acute cases, however, it aggravates the pain. The best results are obtained in cases where diathermy or iontophoresis have diminished the pain but failed to abolish it entirely. The current, administered in a full-length bath, may be used in cases of hyperpiesia, but treatment by general diathermy is usually more reliable.

"Currents which are interrupted or reversed more frequently than once or twice per second should not be passed through the body just as they are, even if the maximum strength they attain is easily borne by the patient. It is obvious that muscles on the path of the current will remain in a state of clonic or Ionis contraction if the periods of rest between the stimuli are too short. Prolonged contraction without rest soon fatigues the muscles, and harmful results follow. Even if there is no actual contraction of muscle, there should be periods of rest between those of stimulation, because biological changes occur in response to electrical excitation of muscle and nerve. Although there is a period of rest between each period of flow in the case of some of the interrupted and alternating currents (e.g., the faradic and Leduc currents) it is far too short to allow the muscles to relax before the next stimulus." (Cumberbatch).

To attain this object, the metronome interrupter may be used. A more satisfactory method is to make the current *surge*. The current is made slowly to wax and wane, gradually rising from zero to maximum and gradually falling from maximum to zero. After each complete surge there should be a period of rest before the next surge begins. The duration of each surge should be from one to two seconds, and that of each period of rest should be the same. For this purpose the apparatus constructed at the suggestion of the late Dr. Lewis Jones for attachment to the rotary converter of the Pantostat is very suitable. Tap water in the conical glass is used as the varying vessel resistance. This device is satisfactory for work in private, where one patient is treated at a time. For hospital work it is more customary to use an apparatus for procuring surging sinusoidal alternating current from the main by means of a transformer with a movable secondary coil. The secondary coil suspended by a cable is made to rise and fall over the primary by means of a small electric motor.

Electrodes. The current rhythmically varied should be administered by way of electrodes when local treatment is to be given. These electrodes are similar to those employed for iontophoresis through the skin. They should be of large size, sufficient to cover the whole part requiring treatment. If there is no instrument at hand for producing rhythmic variation, a certain degree can be effected by moving one of the electrodes that convey the current into the body rhythmically over the skin of the area to be treated, and lifting it off at the end of each stroke. In this method the active electrode should not be less than one third of the area over which it is moved.

If the extremities or the entire body are to be treated, electric baths may be used. These are of two kinds—the Schnee bath and the full-length bath. The water in the bath with the metal or carbon introduced into it constitutes the electrode.

The currents for use in the Schnee baths. The current from the main, using a static transformer when the main current is alternating, or a motor generator when it is direct. If there is no main supply at hand, the current from a battery of accumulators may be taken to the motor generator. The sinusoidal current is almost always used when patients are treated in the Schnee baths. The current used in the full-length bath should always be the rhythmically varied sinusoidal. The galvanic current derived from the main should never be used because the risk to the patient from "earth shocks" is too great.

Electrically Induced Convulsions. A method of treating mental diseases by convulsions induced by the passing of an electric current through the brain was originally described by Cerletti and Bini (see also L. B. J. Kalinowsky, *Lancet*, ii/1939, 1232; G. W. Fleming *et al.*, *ibid.*, ii/1939, 1353; R. E. Hemphill and W. G. Walter, *J. ment. Sci.*, 1941, 82, 256). This method has recently replaced the use of leptazol and other convulsive drugs, since by the electrical method the dose can be regulated more accurately, fits induced with more certainty, and the unpleasantness of the older methods largely abolished.

The apparatus used can be attached to the usual mains, and has a range of voltage from 60 to 160 volts and a duration of exposure from 0.1 to 1.0 sec. The current flowing through the patient's head is about 0.5 to 1.0 ampere. The resistance of a patient's head can be measured by passing a small fixed voltage (0.5 volt A.C.) and reading the resistance in ohms on a meter. The resistance is variable and is no guide to amount of current necessary to produce a convulsion. The electrodes are usually made of lead, padded with sponge, which is soaked in saturated saline, and are attached to the head by a thick rubber band. The scalp, at the points of application, is swabbed with spirit in which some saturated saline is dissolved in order to remove the grease film and replace it by a conducting solution. The electrodes are placed over the frontal poles, in front of the coronal suture and above the orbito-meatal line. As the minimum current necessary to produce a convulsion is variable, it is usual to begin a course of treatment with a moderate dose such as 130 volts for 0.2 second, and to increase or decrease this dose on subsequent occasions according to the results produced. If no convulsion is produced the treatment may be immediately repeated at a greater voltage for the same time or at the same voltage for a longer time (e.g., 140 volts for 0.2 second or 130 volts for 0.3 second). The convulsions should occur immediately after the shock and the occurrence of a latent period between shock and convulsions is an indication for an increase in voltage in subsequent occasions. The convulsion is a typical epileptic fit, lasts for 40 to 45 seconds, and is followed by a period of unconsciousness, drowsiness, confusion, and retrograde amnesia. Treatment is usually given twice or three times a week. Patients do not usually regard it as unpleasant.

Treatment is given with the patient lying on a hard couch with a pillow; during the convulsion the arms are held to the sides and the body prevented from assuming a position of hyperflexion. A gag is placed between the teeth to prevent biting of the tongue and the lower jaw held to prevent dislocation. No food should be taken for several hours before treatment and the bladder emptied immediately before treatment.

The following types of mental illness have been treated:—schizophrenia, mania, depression, involutional melancholia, and severe psychoneuroses. Contra-indications are:—acute infections of any kind, tuberculosis, nephritis, uncompensated heart disease, arteriosclerosis, hyperpiesis. Advanced age (if the subject is otherwise healthy) is not a contra-indication. The number of convulsions necessary to produce a remission of symptoms varies from 5–20 to more, and there is a tendency for patients to relapse if treatment is stopped too early. Acute cases often respond well and chronic may show some social improvement. Complications are:—biting of the tongue, dislocating of jaw or other joint, fracture of vertebral bodies or other bones, and an amnesia for recent events which usually disappears in one or two months.

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DIATHERMY

Diathermy is a form of high frequency current in which the oscillations usually vary between $\frac{1}{2}$ and 3 million per second. The circuit consists of a small transformer to raise the mains A.C. supply to about 2000 volts. The rest of the circuit then consists of a condenser, an inductance and a spark gap, the discharges of which initiate the oscillations of current. A valve circuit may be used instead of the spark gap. This type of machine gives a more regular type of oscillation. The patient is usually insulated from the primary circuit by oscillator-resonator coils.

The current is led to the patient by two rubber-covered leads, which should not be more than a few feet long. It may be applied to the patient by lint pads soaked in strong saline solution, or by thin lead or foil plates, closely and evenly applied to the skin. A useful method for the wrists and arms is for the patient to hold a piece of metal tubing in either hand, the leads being attached to these. A useful handle-bar type consists of an ebonite rod about $\frac{1}{2}$ inch diameter and 6 inches long, and the copper cylinders at either end of the same diameter and about 6 inches long.

The chief effect of these currents when passed through the body is the generation of heat in the path of the current. The

amount of heat generated will depend on the current density and resistance of the part. The greater the product of these two the more heat will be generated. The actual rise of temperature will also depend on how the heat is conducted away, and in the case of most of the internal organs, with their abundant blood supply, the rise will not be so great as in less vascular parts. The machine usually has a milliampere meter, but as this does not tell one either the current density or resistance of the part, it is of very limited value, and must not be relied upon. The chief guide is the patient's sensation of pleasant warmth, and it is unsafe to go beyond this or an unpleasant burn may result. These are often comparatively painless, but may take some time to heal. Obviously the treatment should not be given to an anæsthetic area of skin.

Clinically, this property of warming the tissues locally is useful in many conditions, as it stimulates the vascular supply and hence helps the vitality of the tissues. By using large electrodes and large currents the temperature of the body as a whole can be raised (so-called "artificial fever"). By using small metal electrodes a great current density can be produced and tissues can be coagulated and burnt.

Cutting Current. By suitable modifications of the circuit a current is produced that disrupts the tissues, and, using small needle or blade-shaped electrodes, the current can cut the tissues. It is possible to adjust the cut very finely indeed, and so is of use in brain surgery. The cut can also be made so that the edges of the wound are coagulated at the same time to a depth of $\frac{1}{4}$ to 2 mm., and thus the lymphatics and small vessels are sealed. This is of use in some cases of cancer.

Short Wave Therapy. Using even higher frequency oscillations (10—50 million per second, giving a wave length of 30—6 metres), heat effects can be obtained by direct inductance, the patient being placed in the condenser field between two electrodes. With this type of oscillation the metal electrodes need not touch the patient, and this makes the application much easier. Bandages or even clothing need not be removed. Of use in conditions where ordinary medical diathermy would be employed, but deep heating may be a little easier, especially where there are irregular surfaces such as the knee or nasal sinuses. It may also be used to produce therapeutic fever.

The treatment of disease by means of electropyræxia—a review of results in tabes, cerebrospinal syphilis, primary and secondary syphilis, parkinsonian syndrome, multiple sclerosis, chorea, asthma, chronic arthritis, etc.—C. A. Neymann, *Proc. R. Soc. Med.*, 1935, 29, No. 2, 151; see also C. B. Heald, *ibid.*, 171.

The indications for short-wave diathermy are essentially the indications for conventional diathermy. However, it may be shown later that the superior heating ability of certain high frequency apparatus will be effective when conventional diathermy has failed. Further research and careful evaluation of clinical results are required. So far as competent investigators have been able to determine, there is no demonstrable, selective thermal action *in vivo*, nor specific biologic or bactericidal actions that may be attributed to short-wave diathermy. To date, the effects produced can be explained only on the basis of the generation of heat.—J. S. Coulter (Council on Physical Therapy, A.M.A.), *J. Amer. med. Ass.*, i/1936, 214.

Diseases which may rationally be treated by heat respond as well to diathermy as to short-wave treatment, provided they are superficial or not far from the surface, but in the treatment of deeply situated parts of the thorax or abdomen short-wave treatment will prove more efficacious than treatment by diathermy.—E. P. Cumberbatch, *Proc. R. Soc. Med.*, 1937, 30, 213.

It is a fair statement that the application of ultra-short waves has an advantage over the use of classical diathermy. It has a deeper heating effect. Its greatest advantage is apparent when the electrodes in the condenser-field methods are as far removed as possible from the part to be treated, in order to avoid the high-field concentration round the electrodes. For the production of general pyrexia the inductotherm method would appear to offer many advantages. In order to produce any effect on the body, the machine used for short-wave treatment must have a good energy output.—H. J. Taylor, *Proc. R. Soc. Med.*, 1937, 30, 215.

Besides the acute infective conditions, which are the very essence of ultra-short wave therapy, it is far superior to diathermy in three classes of conditions: (1) Gynaecological work, especially in inflammatory tumours of the adnexa. (2) Certain non-inflammatory surgical conditions, e.g., non-inflammatory injuries of bones and soft parts, and as an adjunct to surgical treatment in fractures, dislocation, sprains and especially in injuries to ligaments; it is exceptionally good if there are complications such as thrombophlebitis even in older patients. (3) Some chronic conditions where diathermy is feeble or has failed, e.g., osteo-arthritis of the hip-joint, sciatica in both acute and chronic forms, and spondylitis deformans (in which some extremely good results have been obtained).—J. Wilson, *Proc. R. Soc. Med.*, 1937, 30, 216.

In gonococcal infections and chorea the results were excellent. Some rheumatoid cases showed spectacular improvement—others did not respond. With adequate precautions, the temperature of 106.5° , necessary for the treatment of gonococcal infection, could be maintained for the necessary six hours. Even temperatures of 108.6° had been encountered without serious effect, though it was undesirable to exceed 107° .—Kerr Russell, *Proc. R. Soc. Med.*, 1937, 30, 219.

Short-wave therapy has become definitely established in certain restricted pathological fields as follows: (1) Pyogenic skin inflammations, empyema of the accessory nasal cavities, pleurisy and lung abscesses. Correct dosage, which is still entirely a matter of guesswork, is essential, over-dosage being dangerous and under-dosage ineffective. (2) Various ophthalmological conditions, such as inflammation of the lacrimal duct, iritis, and retrobulbar neuritis. (3) Chronic rheumatic arthritis; here treatment with large dosage is usually necessary; the improvement is often considerably delayed; the most severe forms of poly-arthritis may show astonishing improvement.—Schliephake, First International Short-Wave Congress (Vienna, 1937), *per Lancet*, ii/1937, 707.

CERVICITIS. By a method devised by C. A. Robinson an infected and inflamed cervix uteri can be heated to the maximum temperature desirable, i.e., 115°C. , which will free the cervix of infection by pathogenic organisms. Untreated cervicitis may lead to infective arthritis, the development of which in a woman with cervicitis is indicated by deterioration of general health, undue fatigue on exertion, occurrence of bruises on the body, loss of healthy colour, and muddy complexion. Chronic low back ache is commonly due to cervicitis, and is commonly the prelude to infective arthritis, disappearing after removal of the cervicitis by diathermy. The results with diathermy in cervicitis are among the most satisfactory in the whole realm of therapeutics.—E. P. Cumberbatch, *Practitioner*, ii/1933, 517.

Pyrotherapy, using the Kettering hypertherm, is more convenient for the patient and less exhausting than hot baths or diathermy in the treatment of gonococcal arthritis. Instituted early, a prompt cure can be effected in 80%, and relief in 90%. Cures are reduced to 35% if fever therapy is delayed more than six weeks after the onset of the arthritis.—P. S. Hench, *J. Lab. clin. Med.*, 1936, 21, 524.

The wholesale raising of the temperature of all the tissues by this apparatus is still a procedure greatly in advance of those methods which only cause a local rise of temperature over a limited area. Of 31 patients with acute gonococcal arthritis, the average improvement in joint function immediately after the conclusion of the course of fever therapy was 80%, ultimately in 29 patients the restoration of joint function was complete. Of 14 patients with chronic gonococcal arthritis the ultimate improvement in joint function was 92.8%; in 7 patients all evidence of joint disease disappeared.—W. M. Simpson, *Brit. J. vener. Dis.*, 1936, 12, 133.

Excellent results are obtained with fever therapy in (1) acute gonorrhoea and in many gonorrhoeal complications including arthritis, epididymitis, orchitis, pelvic inflammation and ophthalmia; (2) neurosyphilis in most of its forms; (3) early syphilis when fever is combined with chemotherapy; (4) late syphilis

with symptoms of inflammatory nature, namely, iritis, interstitial keratitis, and periostitis; (5) Sydenham's chorea. There is a diversion of opinion as to its value in (1) infectious or rheumatoid arthritis and other forms of arthritis; (2) chronic asthma; (3) multiple sclerosis. No definite results but interesting experiences are reported in (1) tuberculosis; (2) subacute bacterial endocarditis; (3) malignant tumours. A summary of the literature, with an extensive bibliography.—H. C. Solomon and I. Kopp, *New Engl. J. Med.*, ii/1937, 805.

Most strains of gonococci, particularly those freshly isolated are very susceptible to heat and are killed in less than five hours at a temperature of 106.7°F. and excellent results have been secured in the treatment of resistant cases of gonorrhœa with artificial fever. In most cases one treatment of 5 hours at a temperature between 106° and 107°F. is sufficient to effect a complete cure.—C. A. Janeway, *New Engl. J. med.*, ii/1940, 101.

Electric Shock. There is not the smallest danger of sudden death if the current enters one foot or leg and leaves by the other, but there is danger if a current at only 65 volts travels *through the thorax and so has a chance to pass through the substance of the heart.* A dry skin offers greater resistance to the entrance of electrical current than a moist one.

100 volts is thought to be dangerous—50 may be considered unsafe. The danger depends also on amperage—1/10 ampere would produce death, but, medically, persons have "endured" 1 ampere without fatal results.

The question of danger to man of electric currents may be discussed under six headings: (a) *Voltage.* Death has occurred from shock at voltages as low as 65 with *alternating currents.* A *direct current* at 95 volts has caused death. (b) *Amperage.* 70 to 90 *ma.* of an ordinary alternating current would be enough if the current went through the chest and heart. (c) *Duration of the contact.* (d) *Industrial alternating currents* are, other things being equal, more dangerous than continuous currents (2 or 3 times as powerful.—Board of Trade agrees as to this.) (Alternating current that reverses the direction of its flow 100 times a second is described as an alternating current of 50 *periods* or cycles a second, or as having a frequency or periodicity of 50 cycles). The frequency is of great importance in considering its danger to life. (e) *Position of electrodes.* The heart is the danger point. (f) *Resistance* at the electrodes.

Treatment of Injuries Caused by Electric Currents

In case of shock, only apparent death is produced at first, and it may be possible to resuscitate by artificial respiration if used *at once.* If patient is in contact with the wire pull him away by catching hold of his *clothing* or by using a good thick layer of cloth, e.g., one's coat (*dry*), or by using a newspaper. Do not touch him unprotected—use rubber gloves if available. In any circumstances the breaking of the current means a fresh shock to the individual concerned. If in contact with a live wire this is to be cut, if possible, with long iron scissors in wooden handles.

For treatment of burns, apply boric acid compresses or charcoal poultices if there is much destruction of tissues. A common result of a severe electric shock is rupture of fine vessels in the brain. Hence, in *first aid*, the head should be raised, not lowered.

If a doctor on his arrival finds artificial respiration being practised he should not, because the heart-beat cannot be detected, presume life to be extinct, but should advocate perseverance in artificial respiration. It is clear that the importance of immediate application of artificial respiration in cases of apparent death from electric shock and of persistence in applying it, though generally appreciated by electrical engineers, is not adequately realised by factory occupiers or by members of the medical profession. Both the Silvester and Schafer methods are applicable, the important thing being immediate application, combined with inhalation of oxygen-carbon-dioxide mixture.—Form 1705, Nov. 1933, Factory Dept., Home Office, per *Lancet*, ii/1934, 206.

All cases of electric shock should be treated by artificial respiration immediately after the accident.—S. Jellinck, *Arch. Radiol.*, 1927, 317.

The best explanation of deaths from electric shock is that they are due to a sensory stimulation causing paralysis of the respiratory centre, justifying artificial respiration. Many cases may be only apparent death, real death supervening from lack of means of carrying on the essential functions of the body.—Bernard Spilsbury, *Arch. Radiol.*, 1927, 316.

ACTINOTHERAPY

ULTRA-VIOLET RAYS

Finsen Lamp. This was the first source of ultra-violet light, the concentrated light being violet and ultra-violet. It is produced by an arc lamp in which the heat rays are cut off. Finsen's original lamp was superseded by the "Finsen-Reyn" lamp, and this again has given place to the:

Finsen-Lomholt Lamp, designed for the local treatment of skin lesions, especially skin tuberculosis, with concentrated carbon arc light. Its use is based on the observation that the therapeutic effect of light treatment depends almost entirely on the ultra-violet rays. By using a double colour filter it is possible to absorb not only the dark heat rays but also the luminous rays without reducing effective ultra-violet rays by more than about 20%. By passing the carbon arc light through the filter a mixture of rays is obtained very rich in ultra-violet energy (about 75%), which, with the old apparatus did not amount to more than about 15% of total radiation energy. It is thus possible to carry out a much more intensive radiation without risk of burns, while cutting the radiation time down to about half.—Svend Lomholt, *Lancet*, ii/1933, 1034.

Tungsten Arc Lamp. The amount of ultra-violet radiation obtained from any metallic electrode appears to be directly proportional to the melting-point of the metal. Tungsten has the highest melting-point of any metal obtainable. Tungsten arc electrodes appear therefore to be the most efficient source. Radiations have destructive action on micro-organisms and cause active hyperæmia in superficial tissues. Protection of the eyes is essential.

Failure frequently due to unsuitable or inefficient apparatus. A tungsten arc lamp, capable of carrying 20 amperes, is the most efficient for local or general treatment. The intensity of the electrical energy is important—a 5-ampere tungsten lamp is relatively ineffective compared with a 15-ampere lamp used at a distance of from 12 to 6 inches for local treatment.—W. J. Turrell, *Brit. med. J.*, ii/1932, 174.

The **carbon disulphide lamp** and the **mercury vapour lamp** also produce ultra-violet light; the latter has been developed by P. Cooper Hewitt. Schattner and Kusch enclosed the mercury in tubes of fused rock crystal—thereby obtaining a very strong source of the light.

A resistance coil enables voltage to be adapted to the requirements of the lamp. The eyes and skin must be protected in using by an ordinary sheet of glass.

A **water-cooled quartz mercury vapour lamp** for the treatment of chronic inflammatory diseases of the mouth, pharynx, and larynx, by local application. The lamp emits luminous and ultra-violet rays of wave-length from about 8000 to 2300 A.U., the infra-red and heat rays being absorbed by the water circulation. It operates on an electric current of 1 to 1.5 amps. and 80 to 120 volts between the electrodes (direct current).—A. Eidenow, *Brit. med. J.*, ii/1933, 94.

The proportion of ultra-violet rays emitted by a lamp depends on whether it is water-cooled or not, and also upon the age of the lamp. Ultra-violet radiation is more intense as the temperature of the tube rises.

Penetrating Power of Different Rays. Those with wave-length from 2000 to 2400 A.U. are stopped in the stratum corneum of the epidermis: from 2500 to 3300 are stopped in the stratum mucosum of the epidermis: from 3400 to 3900 pass through the epidermis and are stopped by the blood in the subepidermal capillaries. Visible violet light rays (4000 A.U.) probably penetrate no further than longest ultra-violet light rays, but visible red rays (7900 A.U.) may reach the superficial strata of the muscles under the deep fascia. Visible green and yellow rays have an intermediate, and invisible heat rays a feeble penetrating power.

The human epidermis is permeable to ultra-violet radiation, from 96 to 26% transmission being found for wave-lengths from 437 m μ to 294 m μ respectively.—N. S. Lucas, *Biochem. J.*, 1931, 57.

Effect on Metabolism. Exposure of the skin of animals to ultra-violet radiation gives increased bactericidal power to the blood and serum.

Ultra-violet light has a distinct effect on cell metabolism, this effect being exerted not only locally on the skin but on deeper organs and on the general body metabolism as well.—*J. trop. Med. (Hyg.)*, 1924, 78.

Growth is promoted, it has been said, by breathing air which has been irradiated with ultra-violet light, but Webster and Hill definitely conclude that it has no effect on growth.—*Nature, Lond.*, i/1924, 761.

Ultra-violet light depresses the lipase and stimulates the protease in the blood.—per *J. Amer. med. Ass.*, ii/1925, 66.

Effect on the Skin and Body. The most potent erythema-producing rays are those from 2900 to 3000 A.U.: the pigment-producing rays are those from 2900 to 3300 A.U. Rays below 2900 produce marked erythema without pigmentation. Amongst other important effects are the activation of cholesterol, increase of calcium and phosphorus content of blood, and of red blood corpuscles and haemoglobin, and increased power of the irradiated body to combat infection. It is mainly by indirect action that the rays have therapeutic effect. Only in a very few diseases is local treatment of value and even in these, general treatment is of more value.

The "short" rays (2000 to 2400 A.U.) do not produce any biological effect, as they do not penetrate beyond the dead tissue of the epidermis—if they fell on living tissue they would destroy it to a slight depth. Although the rays 2300 to 2400 A.U. are the most powerfully bactericidal, they have only very feeble penetrating power. The longer the wave-length the lower the germ-destroying power, and therefore ultra-violet rays cannot destroy bacteria by direct action if they be more than the very slightest depth below the surface.

Dosage in Ultra-violet Treatment. Methods of dosage consist of (1) biological standardisation, using *infusoria*; (2) test of sensitiveness of the skin to light; and (3) effect of a test dose on the bactericidal powers of the blood. The bleaching of an acetone solution of methylene blue may be used in place of the *infusoria*.—A. Eidenow, *Lancet*, ii/1925, 317. See also A. Webster and co-workers, *Lancet*, i/1924, 745.

Pastilles of chloralformamide and diphenylamine, though originally white in colour, change to a progressively deepening yellow on exposure to

ultra-violet light. The pastilles are sensitive to all radiations extending between 3800 A.U. and 2300 A.U. The measurement of the tint is carried out with a tintometer. The pastilles are unaffected by diffused light and do not alter in colour for some hours after exposure.—L. A. Levy and D. W. West, *Brit. J. Radiol. (B.A.R.P. Sect.)*, Oct. 1926, 140.

Chemical method for standardisation—the **Uroxameter**, by action of the rays on a solution of oxalic acid and uranium acetate.—*Lancet*, i/1927, 353; see also J. E. Moss and A. W. Knapp, *Brit. J. Actino-Therap.*, 1927; *Brit. chem. Abstr. (A)*, 1927, 322.

Method of Exposure. The patient is divested of clothing (except over genitalia) and lies on his back, with eyes protected by goggles, the lamp hanging 3 feet above middle of body, but a little to one side. First exposure 2 minutes front and back, repeated every other day, increasing exposure each time by $\frac{1}{2}$ minute up to 10 minutes each front and back. This completes course. If longer course necessary, save time by bringing lamp to 2 feet and reducing exposure from 10 to 5 minutes. If erythema occurs stop treatment till it has disappeared. In children, start with 1 minute exposure increased by $\frac{1}{2}$ minute, and in infants $\frac{1}{2}$ minute, increased by $\frac{1}{2}$ minute.

Diseases and patients in which treatment is likely to produce harmful effects. Patients with general pyrexia should not receive it. In acute local infection local rays should not be used, nor generally if body temperature is raised. Omit treatment if pus present or suspected. Should not be given in pulmonary tuberculosis except by an expert. Inadvisable in case of failing heart, Bright's disease, and in very old people. Omit during menstruation.—E. P. Cumberbatch, *Brit. med. J.*, ii/1928, 43.

Deleterious Effects. Severe dermatitis following an artificial sun bath.—H. MacCormac and H. M. McCrea, *Brit. med. J.*, i/1925, 693.

Patients with unduly low blood pressure may be intolerant to ordinary doses and develop lassitude, depression, headache, etc., but will often receive benefit from subminimal doses.—J. B. Ferguson, *Brit. med. J.*, i/1926, 403.

Skin irritation following. In the milder cases bathing the skin with alkaline lotions is effective. A routine examination of the urine is undertaken and in cases showing marked acidity an alkaline mixture is given, and increased exercise in the open air, to prevent irritation which may arise from deficient alkali reserve.—S. van S. Boyd, *Lancet*, ii/1928, 1076.

In addition to radium and X-rays it is now known that ultra-violet light is carcinogenic. Wherever, as in Australia, the Southern United States or the Argentine, people of European extraction live in a semi-tropical environment an excessive amount of cancer of the skin of the face and hands results. The incidence in such countries is always greatest among farmers and others who are most exposed to the weather, while the minimum time of exposure necessary to ensure the development of cancer is in the neighbourhood of fifteen years. It has been found that the carcinogenic action resides in the wave-length between 2900 and 3341A; wave-lengths of 2537 and 3341A and above are non-carcinogenic.—*Lancet*, ii/1941, 193.

GENERAL VIEWS ON ACTINOTHERAPY

Although actinotherapy cannot fulfil all the claims made for it by its most sanguine exponents, there is a definite set of conditions in which its employment is indicated, and though in skilled hands it may prove valuable, improperly used it may do the gravest mischief. Properly applied, it is an important agent in the AMELIORATION OF RICKETS AND SURGICAL TUBERCULOSIS—often producing complete cure—and is of benefit in some NEUROLOGICAL CONDITIONS (e.g., acute anterior poliomyelitis, Bell's palsy, the root pain of tabes dorsalis, and herpes zoster), ANÆMIAS and SKIN DISEASES. But it may do definite and irretrievable harm in pulmonary tuberculosis, arteriosclerosis, chronic nephritis, quiescent appendicitis, and various forms of neurosis. Early or latent phthisis may flare up into activity.—*Brit. med. J.*, ii/1928, 662.

Types of cases likely to benefit by light treatment in a general clinic are (1) tuberculosis of bones, joints, and glands of the peritoneum, and lupus vulgaris of the skin and mucous membranes; (2) rickets; (3) blood disorders; (4) neurasthenia; (5) some forms of chronic arthritis; (6) *B. coli* pyelitis.—J. H. Sequeira and W. J. O'Donovan, *Lancet*, i/1925, 909.

Gratifying results in furunculosis, eczema, alopecia (especially alopecia areata), onychia, chilblains, Raynaud's disease, psoriasis and pruritus, also in disordered

menstruation. Relieves pain in sciatica and lumbago and other forms of fibrositis and neuritis. Its immediate analgesic effect little short of miraculous.—F. H. Humphris, *Practitioner*, i/1926, 380.

A valuable remedy for erysipelas and certain types of cutaneous and sub-cutaneous tuberculosis, also in acne vulgaris, adenoma sebaceum, pityriasis rosea, parapsoriasis, psoriasis, telangiectasis, indolent ulcers and wounds and port-wine stains; in eczema it is likely to do more harm than good, in pruritus it may actually increase the discomfort, and in the disseminate type of neuro-dermatitis it is extremely dangerous. Its real field is in the treatment and prevention of "surgical" tuberculosis.—G. M. Mackee, Council on Physical Therapy of the A.M.A., per *Brit. med. J.*, ii/1932, 67.

There is no scientific reason to suppose that the supply of vitamin D to the body is better effected by ultra-violet rays than by the direct provision of the necessary food values, and it costs three to four shillings to give by light an effective supply of vitamin D that would cost less than a penny if given as cod-liver oil. As to the power of light radiations to excite local inflammatory reactions in the skin this can be effected equally as well by a mustard plaster. It would seem to be the duty of those taking the responsibility of prescribing light treatment not only to secure that its known dangers shall be avoided, but to find and announce evidence of its benefits other than those due to commercial advocacy and popular credulity.—*Rep. med. Res. Coun., Lond.*, 1927-28, *Lancet*, i/1929, 628. Criticisms of the Report: H. S. Banks, *Lancet*, i/1929, 684; M. Weinbren, *ibid.*, 685; G. M. Levick, *Brit. med. J.*, i/1929, 620.

AFFECTIONS TREATED WITH ULTRA-VIOLET LIGHT

ACNE VULGARIS. Local treatment with the mercury vapour lamp may cause temporary clearing of disfiguring blackheads and papules, but relapse occurs speedily and the treatment may be considered disappointing.—W. J. O'Donovan, *Med. Pr.* (Symposium No. 2), 1937, 16.

ALOPECIA AREATA. Local light therapy is useless and no more than a local rubefacient.—W. J. O'Donovan, *Med. Pr.* (Symposium No. 2), 1937, 16.

ALOPECIA TOTALIS treated with ultra-violet rays. Cases responded well, and recovered growth of hair.—A. Eidenow, *Brit. med. J.*, ii/1930, 940.

ANALGESIC EFFECT. Radiant heat and ultra-violet light are both powerful.—F. Hernaman-Johnson, *Practitioner*, i/1926, 319.

ASTHMA resistant to all other treatment cured by ultra-violet light: three cases quoted. Results probably due to leucocytosis, germicidal action, increased absorption of calcium and phosphorus and formation of vitamin D, increase of iron in the blood, rise of hæmoglobin in the erythrocytes, and increased secretions of thyroid and adrenal glands.—A. Bryce, *Brit. med. J.*, i/1927, 510. The number of attacks is considerably reduced, but relapses occur in two-thirds of the cases.—*Brit. med. J. Erit.*, i/1927, 54.

Infantile asthma well treated. Caution needed owing to production of ozone by quartz lamp with irritant effect on bronchi and lungs.—*Brit. med. J. Erit.*, i/1926, 52.

Good results in 12 cases.—G. H. Day, *Brit. med. J.*, i/1936, 8.

ECZEMA. 100 cases in the Finsen Institute at Copenhagen well treated by the application of concentrated light from a carbon arc lamp. Single exposure for each spot 70 to 140 minutes of a carbon arc light of 50 amperes and 55 volts. As it is laborious and expensive, its use should be limited to resistant cases.—Svend Lomholt, per *J. trop. Med. (Hyg.)*, 1923, 202.

ERYSIPELAS. Erysipelas responds in a wonderful way to light therapy. The eyes are shielded and the patient is given two minutes of a full-sized mercury vapour lamp at a distance of two feet. After four days the skin is flat and peels in large flakes and the skin below is of natural texture and healthy. Cannot conceive a quicker, safer or more reliable therapy.—W. J. O'Donovan, *Med. Pr.* (Symposium No. 2), 1937, 16.

EYE AFFECTIONS. Ocular tuberculosis in any form gives ready response. Phlyctenular ophthalmia also well treated. Infective irido-cyclitis cases show less dramatic response.—W. Stewart Duke-Elder, *Brit. med. J.*, i/1926, 891.

FRACTURES. Especially where there is delay in union, a combination of ultra-violet light with direct current proved effective.—C. B. Heald, *Lancet*, i/1925, 162.

IMPETIGO CONTAGIOSA. Slight but definite curative effect. General exposure in this complaint safer and more efficient than local exposure at short range.—J. B. Ellison, *Lancet*, i/1927, 1345.

In cases of the ordinary crusted impetigo of the face a cure can be promised in five days if the patient will arrange to be treated as for erysipelas (*q.v.*).—W. J. O'Donovan, *Med. Pr.* (Symposium No. 2), 1937, 16.

LUPUS and other forms of tuberculosis.—A. Reyn, *Brit. med. J.*, ii/1923, 499. 90% of cures obtained in lupus vulgaris and other forms of skin tuberculosis with combined treatment locally and the light bath.—*Lancet*, ii/1923, 511.

Carbon arc light in lupus. 70% of cures in the dry type at London Hospital by Finsen's method.—J. H. Sequeira, per *J. trop. Med. (Hyg.)*, 1923, 292. Of little use in lupus erythematosus.—*Brit. med. J.*, ii/1924, 514.

Extensive lupus cleared out completely in less than 3 months, using the Finsen-Lomholt lamp.—E. M. Holmes, *Lancet*, ii/1933, 1033.

The treatment is of great value in improving the condition of the patients, both as regards the general state of health and the activity of the local lesion, and is most effective in those cases which are classified as catarrhal lupus. Efficient treatment, however, necessitates long-continued and regular attendance. It happens not infrequently that the catarrhal element in an area of lupus is largely removed and the lesion resolved into a nodular type, but the beneficial effect stops there.—Alex Maclean, *Edinb. med. J.*, 1937, 256.

NEURALGIA following herpes zoster well treated with ultra-violet rays.—*Prescriber*, 1926, 138.

NOSE AND THROAT diseases treated.—A. Eidenow, *Brit. med. J.*, i/1929, 289.

Tuberculous and other buccal pyogenic chronic ulceration, rapid healing.—A. Eidenow, *Lancet*, ii/1929, 651.

PARALYSIS, INFANTILE. Good results with light treatment in conjunction with local treatment of the affected muscles by red rays.—G. Murray Ievick, *Lancet*, i/1925, 686.

PSORIASIS. The combined treatment of psoriasis with crude coal tar ointment and exposure to ultra-violet quartz lamp better than either treatment alone. The ointment is applied to patches for 24 hours and removed with olive oil. The light is applied for one minute at a distance of 30 inches, and the time increased one minute daily for 3 or 4 days.—per *J. Amer. med. Ass.*, ii/1925, 226.

Uniformly good results obtained by the following method. Two per cent. crude coal-tar ointment in soft paraffin is rubbed into all lesions each night. In the morning, with the ointment still on, the patient goes to the ultra-violet radiation department, where the ointment is removed by means of olive oil, leaving a thin film, then ultra-violet irradiation is given. This treatment is given every day, the patient taking 1000 units of vitamin B₁ daily. This treatment was successful in every one of 53 cases.—A. Bigham, *Brit. med. J.*, i/1941, 692.

PYORRHOEA with systemic infection. Erythema dose of ultra-violet rays administered to trunk, a specific cure.—*Brit. med. J. Erit.*, ii/1926, 101.

Beneficial results by use, in conjunction with ultra-violet rays, of 1% eosin in pyorrhœa alveolaris, of 5% protargol in skin diseases, and of saline in tuberculosis and rickets.—G. Matteucci, *The Limitations and Defects of Actino-Therapy*.

RHEUMATIC DISEASE (chronic). Beneficial. Local analgesic powers considerable in neuritis, fibrositis, and arthritis.—A. G. Watson, *Prescriber*, 1926, 412.

RICKETS. The rays the most active and most powerful treatment in early childhood.—*Brit. med. J. Erit.*, i/1925, 8. See also F. H. Humphris, *Lancet*, i/1925, 912.

Rickets treated with artificial sunlight reinforced by administration of eosin.—*J. Amer. med. Ass.*, i/1926, 1407. (1 grain doses have been used.)

TINEA TONSURANS. Light therapy is useless and unjustifiable.—W. J. O'Donovan, *Med. Pr.* (Symposium No. 2), 1937, 16.

TUBERCULOSIS. A valuable adjunct to other treatment.—H. Godde, *Lancet*, ii/1923, 238. Whole body exposed gradually to the light from short flame carbon arc lamps consuming 75 amps. For patients who are receiving maximum dosage of 2 or 2½ hours it is not economical.—G. B. Dixon, *Brit. med. J.*, ii/1925, 473.

Review of treatment of peritoneal and glandular tuberculosis in children by ultra-violet rays during last few years. The authors conclude that (1) the sole use of ultra-violet rays has been of decided value in peritoneal, glandular and osseous tuberculosis, (2) mesenteric glandular tuberculosis is the most rapidly improved, then mediastinal and lastly peripheral glandular tuberculosis, (3) pulmonary miliary tuberculosis, even in early stage, is unaffected.—*Brit. med. J. Erit.*, i/1925, 3.

All forms of tuberculosis, except pulmonary and meningococcal, in which it is contraindicated, are benefited—fresh air an important adjunct.—*per J. Amer. med. Ass.*, ii/1925, 1091.

The original idea that artificial heliotherapy would prove an almost specific treatment for surgical tuberculosis has not been justified. A series of cases thus treated showed no marked improvement over those not so treated. Artificial heliotherapy does not change the fundamental principles of treatment of surgical tuberculosis.—E. C. Mekie, *Brit. med. J.*, ii/1928, 243.

Genito-urinary tuberculosis: of value. Treatment lasts for 2 years.—*Brit. med. J. Erit.*, i/1925, 12.

TUBERCULOUS LARYNGITIS at Copenhagen; disease arrested in 50% of cases.—*Lancet*, ii/1923, 512; O. Strandberg, *Lancet*, ii/1923, 1237.

Treatment by the Kymayer model No. 2 water-cooled mercury arc vapour lamp, with a quartz laryngeal applicator designed by Dr. William Beaumont, shows promising results, but is probably best employed as an adjuvant to other methods of treatment.—R. Scott Stevenson, *Brit. med. J.*, ii/1933, 964.

Results of light treatment, using the carbon arc lamp, on laryngeal tuberculosis at the Vejlebjerg Sanatorium (Denmark). Percentage of healing in 257 patients, 59.4%. The prognosis of a laryngeal lesion as a complication of phthisis is no longer to be considered so gloomy as hitherto supposed. A great majority can be helped by a combination of light treatment, endolaryngeal intervention, and sanatorium régime, including all the available methods of active lung treatment.—O. Strandberg and J. Gravesen, *Lancet*, i/1934, 128.

VARICOSE ULCERS. During the past four or five years over 240 cases of varicose ulcers have been treated and over 90% have been healed within 6 months by the following technique. The size and nature of the ulcerated area of skin is recorded by direct tracing over a piece of transparent tissue paper with a dermatograph pencil. This tracing is transferred to a sheet of foolscap paper, and the area of unhealed skin recorded week by week, so that the nature and rapidity of healing can be observed. The leg and ulcerated area is then cleansed with acetone and oil of eucalyptus. A piece of tissue crepe paper is then applied to cover the whole leg. The paper covering the ulcer and the skin surrounding this for 1 to 1½ inches is cut away and pieces of adhesive strapping applied to support the paper covering the healthy skin of the leg and foot. The ulcer area and surrounding skin is then exposed to the rays of a vacuum type of quartz air-cooled mercury vapour lamp, consuming 2.5 amps. and 105 volts between the electrodes, at a distance of 12 inches for 10 minutes. This is equivalent to five times the erythema dose of the normal white skin. Immediately after exposure the tissue paper is removed and an elastic adhesive bandage tightly applied from the base of the toes, over the foot including the heel, and up the leg to the knee. The bandage is kept on for a week without interference and is then removed, the skin cleansed with acetone, and the treatment repeated. The ulcer area becomes smaller week by week until healing ultimately occurs. At the final stages of healing the elastic bandage may be discarded and a milder dose of ultra-violet rays applied to the skin of the whole leg and foot, e.g., exposure at a distance of 20 inches for 5 minutes. The patient bathes the limb daily in warm water and oatmeal, and, after drying, olive or almond oil is applied. A similar technique is applied in varicose eczema. Varicose veins are treated by injection as soon as the ulcer or eczema is healed.—A. Eidenow, *Brit. med. J.*, i/1937, 17.

Sunlight. No artificial source of radiation yet found which has a spectral energy distribution exactly like that of sunlight; that of the carbon arc is the closest approach, but even that contains ultra-violet light of very short wavelengths and infra-red radiation of long wave-lengths not found in sunlight; it also emits an intense violet radiation in excess of that in sunlight.—*J. Amer. med. Ass.*, i/1929, 836.

The importance of skyshine: the sky is the most valuable source of ultra-violet rays. Bright clouds and blue sky give more ultra-violet radiation than the high sun and far more than the low sun.—Leonard Hill, *Brit. med. J.*, i/1928, 618.

Sunlight and artificial sunlight. Influence on health. Owing to smoke pollution in cities, the ultra-violet rays are cut down by half, and even two-thirds, in comparison with country and seaside places.—L. Hill, *Brit. med. J.*, ii/1925, 471.

The Acetone-Blue Gauge shows that on the average two-thirds of the ultra-violet rays are cut off by smoke and dust pollution of the atmosphere in the City of London.—*Rep. med. Res. Coun., Lond.*, 1925-6, *Lancet*, i/1927, 508.

Sun Cure of Tuberculosis. Good effects from the sun cure of tuberculosis can only be obtained by means of exact medical observation and supervision. The patient should be slowly acclimatised to the sunshine, starting with 5 minutes exposure of the legs only, and slowly increasing daily until in 12 days a complete sun-bath of an hour's duration is allowed. Results of treatment remarkably good. In unfavourable weather artificial sunlight is given.—*Lancet*, ii/1923, 237.

The only untoward result seen in light-bath treatment is the occasional "flare up" of a tubercular process, especially where there is pyrexia. Wise to begin treatment of visceral tuberculosis with very short exposures limited to small areas. It is generally accepted that patients whose skin pigments best make the most rapid and complete recoveries, though the pigmentation is probably only an index of some chemical change in the blood.—J. H. Sequeira, *Brit. med. J.*, ii/1924, 515.

When undertaken not under medical advice, insolation is suitable only for the well man who feels the better for it. For the sick and infirm it should never, under any circumstances, be undertaken except under medical supervision. The sun cure should be regarded merely as an adjuvant treatment in non-pulmonary tuberculosis. While advocating the treatment, the author utters a warning that it must be wisely and carefully employed—gradual exposures are essential.—Sir H. J. Gauvain, *Brit. med. J.*, ii/1924, 234.

The dangers of misapplied sun-cures.—Lennox Wainwright, *Practitioner*, ii/1924, 197.

Benefits are undoubtedly obtained by patients suffering from tuberculosis of the bones, articulations, peritoneum, intestine, lymph nodes and larynx when the entire body is exposed to carefully graded doses of natural sunlight or to radiation emitted by certain artificial sources of light rays. The beneficial results of such irradiation are due not only to ultra-violet rays. The visible and infra-red rays, as well as the conditions of the atmosphere, play a certain part in the therapeutic effect.—E. Mayer, Council on Physical Therapy, A.M.A., *J. Amer. med. Ass.*, ii/1935, 1599.

Sunbathing. Details of 11 cases, with the onset or exacerbation of symptoms of pulmonary tuberculosis following sunbathing. It is dangerous for anyone who has had hæmoptysis to sunbathe until tuberculosis of the lungs has been excluded, or for people who have recently lost weight, feel abnormally tired, or have other suspicious symptoms. Sunbathers who feel tired or feverish, or perspire at night after a sunbath should take no more sunbaths if their evening temperature is above 99°F.—A. H. Gosse and G. S. Erwin, *Brit. med. J.*, ii/1934, 15.

Vitaglass and "Rest Light" are types of glass which permit the passage of ultra-violet light.

The transparency of glasses to ultra-violet light. The following percentage transmission was obtained. With no cover, 100; with silica 0.11 inch thick, 85.7; with "Vitaglass" 0.065 inch thick, 20.8 to 24.4; with window glass 0.082 inch thick, 3.1; with "Calorex" glass 0.2 inch thick, none; and with non-actinic glass 0.2 inch thick, 0.4. Results bear out the claims made for the materials.—*Brit. chem. Abstr.*, 1927, 323.

By measuring the ultra-violet intensity from the sky-line at the window-sill and comparing the intensity of illumination at the window-sill with that in the centre of the room, it has been found that only 1/120 of the north sky ultra-violet light reaches the middle of the room, i.e., a child would have to sit in this position for 20 hours to receive as much ultra-violet light as he would receive from 2 minutes out of doors in the noon sunlight. Cheaper and more efficient to give children a short noon-day recess than to invest in special window-glass.—*Lancet*, ii/1928, 890.

Experiments at Smethwick for a year on 240 schoolchildren, proved that apart from slight increase in hæmoglobin in children of "Vitaglass" window classes, the benefit was small, the probable reason being that the children had little of their skin exposed. Open-air schools preferable.—per *Lancet*, ii/1929, 398.

"Vitaglass" exposed to sunlight undergoes slight diminution of transparency in shortest wave-lengths for 9 months or less period.—"Vitaglass" Marketing Board, *Lancet*, ii/1929, 690.

In buildings other than those designed specifically for sun treatment, although ultra-violet glass in large, unobstructed windows can admit therapeutic radiation in appreciable quantity, to receive this radiation it is necessary to sit near a window or in the direct rays of the sun.—"Ultra-violet Window Glazing", H. E. Beckett, *Building Research Bulletin*, No. 8.

A Practical Window for Transmitting Ultra-violet Rays from Sunlight. A cheap, practical, and effective window for the purpose may be made from cellophane. The cellophane is reinforced by being sandwiched between two layers of coarse chicken wire affixed to a wooden frame (1 to 2 inch mesh wire being used), and will last for a year. The material allows the shortest (curative) light-waves to pass. Glass used for the purpose loses a lot of its transparency by exposure.—A. H. Pfund, *J. Amer. med. Ass.*, ii/1928, 19.

Windolite. A cellulose and wire netting product allowing ultra-violet rays through freely.

Infra-Red Rays are invisible and are found in the electro-magnetic spectrum between the red rays of the visible and the Hertzian waves. Those of therapeutic value have a wave-length ranging from 7700 Å to 150,000 Å, subdivided into "near" 7700 Å to 15,000 Å, "middle" 15,000 Å to 30,000 Å, and "far" 30,000 Å to 150,000 Å. So-called "radiant heat" is a combination of visible radiation and infra-red radiation comprising rays with wave-lengths of 4000 Å to 30,000 Å, approximately generated by an incandescent filament lamp of high voltage. These lamps are capable of generating a larger output of visible and short infra-red rays than the non-luminous generators which give only the infra-red. Consequently a larger quantity of energy is available for conversion into heat when the radiations are arrested by the skin. It is this heat conducted by the blood stream and lymphatics away from the skin deep into the tissue which has given rise to the theory of greater penetration. Actually, there is no authentic experimental evidence that the short infra-red rays penetrate farther into the tissue than the long infra-red; in fact, the bulk of the evidence is the reverse. It can be stated that purely infra-red rays are more soothing to the patient, whereas the visible rays are more stimulating.

The most obvious physiological effect is erythema; pigmentation may follow repeated exposures but does not appear to have any therapeutic significance. It may produce a temporary leucopenia and care should be taken in anæmic patients. There is a general rise of body temperature dependent on the area irradiated.

The conditions which benefit by infra-red irradiation are limited and it is pre-eminently a treatment of symptoms, particularly of pain, in the relief of which it surpasses any other form of treatment. It is especially valuable in the treatment of backache, painful joints, stiffness and in the early stages of rheumatism.—W. Beaumont, *Practitioner*, ii/1938, 161.

The therapeutic indications for the use of infra-red rays locally are chiefly in the following fields: surgery, following fractures, dislocations, sprains, cicatrices after operating procedures, arthritis when a limited influence on the joints is desired, myositis, neuritis, and circulatory disturbances of the extremities, such as Raynaud's disease.—*Council on Physical Therapy, A.M.A., J. Amer. med. Ass.*, ii/1934, 27.

Therapeutic Uses of Infra-Red Rays by W. Annandale Troup (The Actinic Press, Ltd.).

"Grenz" or "Infra-Röntgen" Rays, a new form of actinotherapy. The rays are situated between the ultra-violet and Röntgen rays and have a wave-length of 1.2 to 2 Angstrom Units. One unit of "Grenz" rays produces a mild erythema from 12 to 24 hours after exposure: redness disappears in 10 days and dose can be repeated every 2 weeks for several doses. Most diseases require doses of 2 units. Special low-tension apparatus ranging between 5000 and 9000 volts necessary. Of value in skin diseases.—*per Prescriber*, 1928, 321.

GLOSSARIES

DUTCH GLOSSARY

- Aanhoudend*, constantly.
Aanhoudende pijn, ache.
Abces, abscess.
Acht, eight.
Achter, behind.
Achtereenvolgend, consecutive.
Achttien, eighteen.
Achtmaal, eight times.
Achtste, eighth.
Adem, breath.
Ader, vein.
Aetherische olie, essential oil.
Als, if.
Alkohol, alcohol.
Amandel, tonsil.
Anderszins, otherwise.
Arm, arm.
Avond, evening.
Avondeten, supper.
Azijn, vinegar.
Bad, bath.
Balsem, balsam.
Been, bone, leg.
Beide, either.
Bekend, known.
Beurt om beurt, alternately.
Bij, by.
Blaar, blister.
Blaas, bladder.
Bloed, blood.
Bloedstelpend, styptic.
Borst, breast, chest.
Braakmiddel, emetic.
Brandspiritus, methylated spirit.
Capsule, capsule.
Clysteerspuit, enema.
Dag, day.
Dagelijks (adv.), daily.
Dagelijksch (adj.), daily.
Dan, then.
Der, den, the.
Deel, part.
Dekken, to cover.
Den volgenden dag, on the day after.
Derde, third.
Dertien, thirteen.
Dertig, thirty.
Dessertlepelvol, dessertspoonful.
Diëet, diet.
Dij, thigh.
Dikwijls, often.
Door, by.
Dosis, dose.
Drie, three.
Driemaal, thrice.
Driemaal per dag, three times a day.
Driemaal's daags, three times a day.
Drinken, to drink.
Druppel, drop.
Dubbel, double.
- Duizend*, thousand.
Een, one.
Eenmaal, once.
Eens, once.
Eenig, only.
Eerste, first.
Eetlepel, tablespoon.
Eenvoudig, simple.
Elf, eleven.
Elfde, eleventh.
Elixir, elixir.
Elk, elken, every.
Emulsie, emulsion.
Endelarm, rectum.
Enkel, single.
Etiquetteeren, to label.
Evenveel, equal parts.
Exemplaar, copy.
Flesch, vial.
Gebruik, use.
Gedurende, during.
Gedurende den dag, during the day.
Gedurig, frequently.
Geen (adj.), no.
Geheel, whole.
Gelegen, convenient.
Gelei, jelly.
Gelijk, like.
Geneesmiddel, medicine.
Genoegzaam, sufficient.
Getal, number.
Geven, to give.
Gewricht van de hand, wrist.
Glas, glass.
Glasvol, glassful.
Goed, good.
Gom, gum.
Gorgeldrank, gargle.
Gorgelen, to gargle.
Groot, big, large.
Groot drinkglas, tumblerful.
Haar, hair.
Half, half.
Hals, neck.
Hand, hand.
Hard, hard.
Hars, resin.
Hart, heart.
Hebben, to have.
Heden, to-day.
Heet, hot.
Hemzelf, himself.
Herhalen, to repeat.
Heup, hip.
Hoest, cough.
Honderd, hundred.
Honig, honey.
Hoofd, head.
Huid, skin.
Hydrophilegaas, absorbent gauze.

Dutch Glossary—continued

Ieder, every.

Ieder vierde uur, every four hours.

Iets, some.

In, in, into.

Inademen, to breathe.

Inademing, inhalation.

Inblazing, insufflation.

In den nacht, at night.

Indien het hoesten lastig, when the cough is troublesome.

In eenig geval, in any case.

Ingewanden, bowels, intestines.

In plaats van, instead of.

Inspuiting, injection.

Inspuiting in de aderen, intravenous injection.

Inspuiting in de spieren, intramuscular injection.

Inwendig, internally.

Inwrijving, embrocation.

Kaak, cheek.

Kakebeen, jaw.

Keel, throat.

Kinderpapelel, dessertspoon.

Klaar, clear.

Klein, little, small.

Knie, knee.

Knieschijf, kneecap.

Koel, cool.

Koepokstof, vaccine.

Koffielepel, coffeespoon.

Kook, boiling.

Kopje, cup.

Kopjevol, cupful.

Korrel, granule.

Koude, cold.

Kuit, calf.

Kwartier, quarter.

Laatst, last.

Langzaam, slowly.

Laten droppelen, to drop.

Laxeerend, laxative.

Laxeermiddel, aperient.

Lepelvol, spoonful.

Lever, liver.

Lichaam, body.

Licht, light.

Lid, limb.

Links, left.

Lip, lip.

Long, lung.

Maag, stomach.

Maat, measure.

Maken, to make.

Malen, to triturate.

Meer, more.

Melk, milk.

Mengen, to mix.

Mengsel, mixture.

Menig, many.

Met, with.

Meten, to measure.

Met water, in water.

Middag, midday, at noon.

Middagmaal, lunch, dinner.

Middelertwyl, in the meantime.

Midden, middle.

Minuut, minute.

Mond, mouth.

Mondspoeling, mouthwash.

Morgen, tomorrow, in the morning.

Na, after.

Na den maaltijd, after meals.

Nacht, night.

Nacht en ochtend, night and morning.

Nader, near.

Nagel, fingernail, toenail.

Namiddag, afternoon.

Negen, nine.

Negende, ninth.

Negentien, nineteen.

Negentig, ninety.

Nemen, to take.

Neus, nose.

Neusgat, nostril.

Nier, kidney.

Nieuw, new.

Noodzakelijk, necessary.

Nu en dan, occasionally.

Ochtend, morning, in the morning.

Of, or.

Olïe, oil.

Om den anderen dag, every other day.

Om het andere uur, every other hour.

Onder, under.

Onderbuik, abdomen.

Onderhuidscbe inspuiting, subcutaneous injection.

Ongeveer, nearly.

Onmiddellijk, immediately.

Ons, ounce.

Ontbijt, breakfast.

Oog, eye.

Ooglid, eyelid.

Oogzalf, eye ointment.

Oogwater, eye lotion.

Oor, ear.

Op, on.

Op de gebruikelijke wijze, in the usual manner.

Op de voornoemde wijze, as above.

Oplossen, to dissolve.

Oplossing, solution.

Over, over.

Overvloed, plenty.

Overvloedig, copious.

Pap, poultice.

Papelel, dessertspoon.

Pasta, paste.

Pastille, lozenge.

Penseel, brush.

Penseelvocht, paint.

Per gewicht, by weight.

Pijn, pain.

Pil, pill.

Pisbuis, urethra.

Pleister, plaster.

Poeder, powder.

Pols, pulse.

Recht, right.

Dutch Glossary—continued

Rein, pure.
Reuzel, suet.
Rib, rib.
Rood, red.
Room, cream.
Rug, back.
Ruggegraat, spine.
Ruimte, capacity.
Samentrekkend middel, astringent.
Sap, juice.
Schedel, scalp.
Scheede, vagina.
Schouder, shoulder.
Schouderblad, shoulderblade.
Schudden, to shake.
Serum, serum.
Siroop, syrup.
Slagader, artery.
Sleutelbeen, collarbone.
Slijmerig vocht, mucilage.
Slikken, to swallow.
Smakelijkheid, flavour.
Smeersel, liniment.
Snede, cut.
Snel, quickly.
Snijden, to cut.
Somtijds, sometimes.
Spiritus, spirit.
Spoedig, soon.
Staken, to leave off.
Sterk, strong.
Stellen, to place.
Stop, stopper.
Stoven, to foment.
Strottenhoofd, larynx.
Suiker, sucrose.
Tablet, tablet.
Tand, tooth.
Tandvleesch, gums.
Te, at.
Teen, toe.
Tegengift, antidote.
Tenzij, unless.
Thee, tea.
Theelepelfvol, teaspoonful.
Tien, ten.
Tienmaal, ten times.
Tijd, time.
Tijd om naar bed te gaan, bedtime.
Tinctuur, tincture.
Toepassen, to apply.
Tong, tongue.
Tot, until.
Traan, eyedrop.
Trapsgewijs, gradually.
Trekken, to infuse.
Tusschen, between.
Twaalf, twelve.
Twee, two.
Tweede ontbijt, lunch.
Tweemaal, twice.
Tweemaal per dag, twice a day.
Tweemaal's daags, twice a day.
Twintig, twenty.
Uitlaten, to leave out.

Uittreksel, extract.
Uitwendig, external.
Uur, hour.
Van, from.
Van dag tot dag, from day to day.
Van elk, of each.
Van tijd tot tijd, from time to time.
Varkensvrezel, lard.
Veel, much.
Veertien, fourteen.
Veertig, forty.
Vel, skin.
Verband, bandage.
Verdunnen, to dilute.
Vergrooten, to increase.
Verkoelen, to cool.
Verlichten, to relieve.
Vernaling, trituration.
Verzachtend, emollient, sedative.
Vier, four.
Vierde, fourth.
Viermaal, four times.
Viermaal per dag, four times a day.
Vijf, five.
Vijfde, fifth.
Vijfmaal, five times.
Vijftig, fifty.
Vinger, finger.
Vloeistof, liquid.
Voedsel, food.
Voet, foot.
Vol, full.
Volgens het voorschrift, as directed.
Voor, for, before.
Voorhoofd, forehead.
Voornamelijk, chiefly.
Voorzichtig, cautiously.
Voor uitwendig gebruik, for external use.
Voortzetten, to continue.
Vrij, freely.
Vroeg, early.
Vroeger, former.
Warm, warm.
Waschmiddel, lotion.
Wasschen, to bathe.
Water, water.
Waterig, aqueous.
Watten, cotton wool.
Week, week.
Wenkbrauw, eyebrow.
Wijl, whilst.
Wijn, wine.
Wijnglasvol, wineglassful.
Wrijven, to rub in.
Zacht, soft.
Zalf, ointment, salve.
Zelfde, same.
Zenden, to send.
Zenuw, nerve.
Zes, six.
Zesde, sixth.
Zesmaal, six times.
Zestien, sixteen.
Zestig, sixty.

Dutch Glossary—continued

Zetpil, suppository.
Zeven, seven.
Zevende, seventh.
Zevenmaal, seven times.
Zeventien, seventeen.
Zeventig, seventy.
Ziften, to sift.
Zijde, side.
Zijn, his.
Zonder, without.
Zoo, if.

Zoo dikwijls als, as often as.
Zoodra als mogelijk, as soon as possible.
Zoo noodig, if necessary.
Zorgvuldig, careful, carefully.
Zout, salt.
Zuiver, clean.
Zuiverend middel, cathartic.
Zuur, acid.
Zwaar, heavy.
Zwelling, swelling.
Zweeten, to perspire.

FRENCH GLOSSARY

A, to, into, at.
Abscès, abscess.
Abdomen, abdomen.
Abondant, copious, plenty.
Acide, acid.
Agiter, to shake.
Agitez le flacon, shake the bottle.
Alcool, alcohol.
Alternativement, alternately.
À moins de . . . ne, unless.
Ampoule, ampoule.
Amygdale, tonsil.
Antidote, antidote.
Antiseptique, antiseptic.
Après, after.
Après les repas, after meals.
Après-midi, afternoon.
Aqueux, aqueous.
Aromatique, aromatic.
Artère, artery.
Assez, sufficient.
Astringent, astringent.
Aucun (adj.), no.
Au-dessus de, over.
Augmenter, to increase.
Aujourd'hui, to-day.
Au lieu de, instead of.
Au moyens de, by means of.
Au poids, by weight.
Autrement, otherwise.
Avaler, to swallow.
Avant, before.
Avec, with.
Avec empressement, promptly.
Avec précaution, cautiously.
Avoir, to have.
Bain, bath.
Bain de pieds, footbath.
Bande, bandage.
Baume, balsam.
Beaucoup de, many.
Bien, well.
Bientôt, soon.
Boire, to drink.
Bras, arm.
Bouche, mouth.
Bouchon, stopper.
Bougie de l'oreille, aural bougie.
Bougie de l'urètre, urethral bougie.

Bougie du nez, nasal bougie.
Bouillant, boiling.
Bouteille, bottle.
Cachet, cachet.
Capsule, capsule.
Cataplasme, poultice.
Cathartique, cathartic.
Cent, hundred.
Cérat, cerate.
Cesser, to leave off.
Cerveau, brain.
Chacun, chaque, each, every.
Chaque quatrième heure, every four hours.
Chaud, hot, warm.
Cheveux, hair.
Cinq, five.
Cinq fois, five times.
Cinquante, fifty.
Cinquième, fifth.
Clair, clear.
Clavicule, collarbone.
Cœur, heart.
Collyre, eyelotion.
Comme, like.
Confection, confection.
Constamment, constantly.
Continuer, to continue.
Continûment, continuously.
Convenable, convenient.
Corps, body.
Côte, rib, side.
Cou, neck.
Coup, cut.
Couper, to cut.
Couvrir, to cover.
Crème, cream.
Cribler, to sift.
Cuillerée, spoonful.
Cuillerée à café, teaspoonful.
Cuillerée à dessert, dessertspoonful.
Cuillerée à soupe, tablespoonful.
Cuisse, thigh.
Dans, in, into, at.
D'avance, previously.
De, from, of.
De bonne heure, early.
Décoction, decoction.
Degoutter, to drop.

French Glossary—continued

- D'heure en heure*, every hour, hourly.
Déjeuner, breakfast.
De jour en jour, from day to day.
De la façon habituelle, in the usual manner.
De la façon prescrite, as directed.
De l'un de l'autre, of each.
Demain, to-morrow.
Demi (adj.), half.
Dépilatoire, depilatory.
De près, nearly.
Dernier, last.
Derrière, behind.
De temps en temps, from time to time.
Deux, two.
Deux fois, twice.
Deux fois par jour, twice a day.
Deuxième, second.
Diluer, to dilute.
Dîner, dinner.
Dissoudre, to dissolve.
Diurétique, diuretic.
Diviser, to divide.
Dix, ten.
Dix fois, ten times.
Dixième, tenth.
Dix-huit, eighteen.
Dix-neuf, nineteen.
Dix-sept, seventeen.
Doigt, finger.
Donner, to give.
Dos, back.
Dose, dose.
Double, double.
Douleur, pain.
Doux, soft.
Douze, twelve.
Droit, right.
Dur, hard.
Eau, water.
Électuaire, electuary.
Élixir, elixir.
Embrocation, embrocation.
Émétique, emetic.
Emollient, emollient.
Emplâtre, plaster.
Emulsion, emulsion.
En, at, in.
Enème, enema.
Enflure, swelling.
En plein midi, at noon.
En tout cas, in any case.
Entre, between.
Envoyer, to send.
Épaule, shoulder.
Épine dorsal, spine.
Essence, essence.
Estomac, stomach.
Étiqueter, to label.
Être en effervescence, to effervesce.
Exemple, copy.
Extérieur, external.
Extrait, extract.
Faire, to make.
Fiole, vial.
Foie, liver.
Fort, strong.
Frictionner, to rub in.
Froid, cool, cold.
Front, forehead.
Gargariser, to gargle.
Gargarisme, gargle.
Gauche, left.
Gelée, jelly.
Gélatine, gelatin.
Gencives, gums.
Genou, knee.
Gomme, gum.
Gorge, throat.
Goût, flavour, taste.
Gouttes, drops.
Gouttes d'œil, eye-drops.
Gouttes d'oreille, ear-drops.
Graisse, suet.
Grand, big, large.
Granule, granule.
Haleine, breath.
Hanche, hip.
Heure, hour.
Huile, oil.
Huit, eight.
Huit fois, eight times.
Huit jours, week.
Huitième, eighth.
Immédiatement, immediately.
Infuser, to infuse.
Infusion, infusion.
Inhalation, inhalation.
Injection, injection.
Injection intramusculaire, intramuscular injection.
Injection intraveineuse, intravenous injection.
Insufflation, insufflation.
Intérieurement, internally.
Intestins, bowels, intestines.
Jéunant, fasting.
Joue, cheek.
Jour, day.
Journalier, daily.
Jus, juice.
Jusqu'à la douleur se soulage, until the pain is relieved.
Jusque, until.
Lait, milk.
Langue, tongue.
La toux pressant, when the cough is troublesome.
Laxatif, laxative, aperient.
Le, la, les, the.
Léger, light.
Le lendemain, on the day after.
Le matin, in the morning.
Lentement, slowly.
Lèvre, lip.
Librement, freely.
Liniment, liniment.
Liquide, liquid.
Loion, lotion.

French Glossary—continued

Lourd, heavy.
Lui-même, himself.
L'un ou l'autre, either.
Mâchoire, jaw.
Main, hand.
Mal, ache.
Matin, morning.
Matin et soir, morning and evening.
Médecine, medicine.
Mélanger, to mix.
Membre, limb.
Même, same.
Mettre, to place.
Midi, midday.
Miel, honey.
Milieu, middle.
Mille, thousand.
Minute, minute.
Mollet, calf.
Moindre, least, less.
Moitié (n.), half.
Mucilage, mucilage.
Narine, nostril.
Nécessaire, necessary.
Nerf, nerve.
Neuf, nine.
Neuvième, ninth.
Nex, nose.
Nombre, number.
Nourriture, food.
Nouveau, new.
Nuit, night.
Oeil, eye.
Omoplate, shoulderblade.
Once, ounce.
Ongle, fingernail, toenail.
Onguent, ointment, salve.
Onguent pour les yeux, eye ointment.
Onze, eleven.
Onzième, eleventh.
Ordonnance, prescription.
Oreille, ear.
Orteil, toe.
Os, bone.
Ou, or.
Par, by.
Part, part.
Pas de (adj.), no.
Pastille, pastille, lozenge.
Paupière, eyelid.
Peau, skin.
Pendant, during.
Pendant la nuit, during the night.
Pendant le jour, during the day.
Pendant que, whilst.
Pessaire, pessary.
Petit, little, small.
Petit déjeuner, lunch.
Petite cuillerée, teaspoonful.
Pied, foot.
Pilule, pill.
Plein, full.
Pleine une tasse, cupful.
Plein un grand verre, tumblerful.
Plein un verre, glassful.

Plein un verre à vin, wineglassful.
Plus, more.
Poignet, wrist.
Poison, poison.
Poitrine, chest, breast.
Potion, draught.
Pouce, thumb.
Poudre, powder.
Pouls, pulse.
Poumon, lung.
Pour, for.
Pour usage extérieur, for external use.
Précédent, former, previous.
Premier, first.
Prendre, to take.
Près, near.
Propre, clean.
Puis, then.
Pur, pure.
Quarante, forty.
Quatre, four.
Quatre fois, four times.
Quatre fois par jour, four times a day.
Quatre-vingt, eighty.
Quatre-vingt-dix, ninety.
Quatorze, fourteen.
Quatrième, fourth.
Quelque, some.
Quelquefois, sometimes.
Qui suit, consecutive.
Quinze jours, fortnight.
Rectum, rectum.
Refroidir, to cool.
Répéter, to repeat.
Résine, resin.
Respirer, to breathe.
Rince-bouche, mouthwash.
Rotule, kneecap.
Rouge, red.
Sang, blood.
Saindoux, lard.
Sans, without.
S'appliquer à, to apply.
Se baigner, to bathe.
Sédatif, sedative.
Seize, sixteen.
Sel, salt.
Semaine, week.
Sept, seven.
Sept fois, seven times.
Septième, seventh.
Sérum, serum.
Seul, only, single.
S'il le faut, if necessary.
Simple, simple.
Sirop, syrup.
Six, six.
Six fois, six times.
Sixième, sixth.
Soigneux, careful.
Soigneusement, carefully.
Soir, evening.
Soixante, sixty.
Soixante-dix, seventy.
Solution, solution.

French Glossary—continued

Son, sa, ses, his.
Soulager, to relieve.
Souper, supper.
Sourcil, eyebrow.
Sous, under.
Souvent, often.
Styptique, styptic.
Suc, juice.
Suivant, next.
Suppositoire, suppository.
Sur, on.
Sur ces entrefaites, in the meantime.
Tablette, tablet.
Tasse, cup.
Teinture, tincture.
Temps, time.
Temps de se coucher, bedtime.
Tête, head.
Tiede, lukewarm.
Tissu, tissue.
Tour à tour, alternately.
Tous les jours, daily.
Tous les deux jours, every other day.
Tout, every, whole.
Toutes les deux heures, every other hour.

Toux, cough.
Transpirer, to perspire.
Treize, thirteen.
Trente, thirty.
Trituration, trituration.
Triturer, to triturate.
Trois, three.
Trois fois, three times.
Trois fois par jour, three times a day.
Troisième, third.
Tympan, eardrum.
Un, une, one.
Une fois, once.
Un quart d'heure, a quarter of an hour.
Urètre, urethra.
Vaccin, vaccine.
Vagin, vagina.
Veine, vein.
Verre, glass.
Vésicatoire, blister.
Vésicule, bladder.
Vin, wine.
Vinaigre, vinegar.
Vingt, twenty.
Vite, quickly.

GERMAN GLOSSARY

Abend, evening, night.
Abendessen, supper.
Abführmittel } purgative, aperient,
Abführungsmittel } laxative
Abschrift, copy.
Acht, eight, eighth.
Achtmal, eight times.
Achtzehn, eighteen.
Achtzig, eighty.
Ader, blood vessel.
Alkohol, alcohol.
Alle, every.
Alle vier Stunden, every four hours.
Alle zwei Stunden, every other hour.
Allmählich, gradually.
Ampulle, ampoule.
An, by, on.
Anders, otherwise.
Anlegen, to apply.
Anstatt, instead of.
Antiseptisch, antiseptic.
Arm, arm.
Aromatisch, aromatic.
Arterie, artery.
Arznei, medicine.
Arzneiverschreibung, prescription.
Atem, breath.
Atmen, to breathe.
Auf, on.
Aufeinanderfolgend, consecutive.
Aufgeben, to leave off.
Aufbrausen, to effervesce.
Aufguss, infusion.
Auflösen, to dissolve.

Aufschütteln die Flasche, shake the bottle.
Auge, eye.
Augenbraue, eyebrow.
Augenlider, eyelids.
Augenwasser, eyewash.
Augensalbe, eye ointment.
Aus, out, out of, from.
Auslassen, to leave out.
Ausserlich, external.
Ausserlich anzuwenden, for external use.
Backe, cheek.
Bad, bath.
Bähen, to bathe, to foment.
Bald, soon.
Balsam, balsam.
Baumwolle, cotton.
Becher, cup.
Bedecken, to cover.
Bei, by.
Bei nacht, at night.
Bett, leg.
Behnruigendes mittel, sedative.
Beständig, constantly.
Binde, bandage.
Bis, until.
Bis die Linderung des Schmerzes, until the pain is relieved.
Bisweilen, sometimes.
Blase, bladder, blister, bubble.
Blut, blood.
Blutstillungsmittel, styptic.
Brechmittel, emetic.
Breiumschlag, poultice.

German Glossary—continued

- Brust*, chest or breast.
Dann, then.
Daumen, thumb.
Denaturierter Spiritus, methylated spirit.
Der, die, das, the.
Derselbe wie vorher, as above.
Des Nachts und des Morgens, night and morning.
Dessertlöffelvoll, dessertspoonful.
Destilliertes Wasser, distilled water.
Diät, diet.
Doppelt, double.
Drei, three.
Dreimal, thrice.
Dreimal des Tages, three times a day.
Dreissig, thirty.
Dreizehn, thirteen.
Dritt, third.
Ein, one.
Einblasung, insufflation.
Eine Viertelstunde, a quarter of an hour.
Einige, some.
Einfach, simple.
Eingeweide, bowels.
Einmal, once.
Einreibung, liniment.
Einreibungsmittel, embrocation.
Einspritzung, injection.
Einspritzung in die Adern, intravenous injection.
Einspritzung in die Muskeln, intramuscular injection.
Einspritzung unter die Haut, subcutaneous injection.
Einzel, single.
Einzig, only.
Eitergeschwulst, abscess.
Elf, eleven.
Elft, eleventh.
Elixir, elixir.
Emulsion, emulsion.
Einweichen, to infuse.
Enthaarungsmittel, depilatory.
Erst, first.
Erweichende mittel, emollient.
Essenz, essence.
Essig, vinegar.
Esslöffelvoll, tablespoonful.
Etikettieren, to label.
Extrakt, extract.
Fasten, fasting.
Finger, finger.
Flasche, bottle.
Fläschchen, vial.
Flüchtige Öle, essential oils.
Flüssiges Paraffin, liquid paraffin.
Flüssigkeit, liquid.
Fortsetzen, to continue.
Frei, freely.
Früh, early.
Frühstück, breakfast.
Fünf, five.
Fünfmal, five times.
Fünft, fifth.
Fünfzig, fifty.
Für, for.
Fuss, foot.
Fussbad, footbath.
Gabe, dose.
Gabelfrühstück, lunch.
Gallerte, Gelee, jelly, gelatin.
Ganz, whole.
Geben, to give.
Gedärm, intestines.
Gegenmittel, antidote.
Gehirn, brains.
Geistig, spirituous.
Gelegentlich, occasionally.
Genug, plenty, sufficient.
Geringst, least.
Geschmack, flavour.
Geschwulst, swelling.
Gewebe, tissue.
Gift, poison.
Gleich, like.
Gleiche Teile, equal parts.
Glied, limb.
Glyzerin, glycerin.
Gross, big, large.
Gummi, gum, rubber.
Gurgel, throat.
Gurgeln, to gargle.
Gurgelwasser, gargle.
Gut, well, good.
Haar, hair.
Haben, to have.
Halb (adj.), half.
 Hälfte (n.), half.
Hals, neck.
Halsmandeln, tonsils.
Hand, hand.
Handgelenk, wrist.
Harnröhre, urethra.
Harntreibendes Mittel, diuretic.
Hart, hard.
Haut, skin.
Häufig, frequently.
Harz, resin.
Heiss, hot.
Herz, heart.
Heute, to-day.
Hinter, behind.
Hirnschalenhaut, scalp.
Höchst, chiefly.
Honig, honey.
Hüfte, hip.
Husten, cough.
Hundert, hundred.
In, in, at, into.
In der gewöhnten Weise, in the usual manner.
Inhalation, inhalation.
Innerlich, internally.
Jede, of each.
Jedenfalls, in any case.
Jeden zweiten Tag, every other day.
Jeder, each, either, all.
Kalt, cold, cool.

German Glossary—continued

Kamelhaarpinsel, camel's hair brush.*Kapsel*, capsule.*Kehlkopf*, larynx.*Kein* (adj.), no.*Kinnbacken*, jaw.*Klar*, clear.*Klein*, little, small.*Kleiner*, smaller.*Klystier*, enema.*Knie*, knee.*Knochen*, bone.*Kollodium*, collodion.*Kopf*, head.*Körnchen*, granule.*Körper*, body.*Kühlen*, to cool.*Kuhpocke*, vaccine.*Langsam*, slowly.*Lanolin*, lanoline.*Latwerge*, electuary.*Lauwarm*, lukewarm.*Leber*, liver.*Letzt*, last.*Licht* (adj.), light.*Lindern*, to relieve.*Liniment*, liniment.*Link*, left.*Lippe*, lip.*Löffelvoll*, spoonful.*Lösung*, solution.*Lunge*, lung.*Machen*, to make.*Magen*, stomach.*Mastdarm*, rectum.*Mass*, measure.*Mehr*, more.*Menge*, quantity.*Messen*, to measure.*Milch*, milk.*Milchzucker*, lactose.*Minute*, minute.*Mischen*, to mix.*Mischung*, mixture.*Mit*, with, by.*Mittag*, midday, at noon.*Mittagessen*, dinner.*Mitte*, middle.*Mittel*, medium, means, remedy.*Mittels*, *mittelst*, by means of.*Mittlerweile*, in the meantime.*Mit Wasser*, in water.*Mixtur*, mixture.*Mund*, mouth.*Mundwasser*, mouthwash.*Mutterkapsen*, pessary.*Nach*, after.*Nach Bedarf*, if necessary.*Nach dem Essen*, after meals.*Nach Gewicht*, by weight.*Nachmittag*, afternoon.*Nächst*, next.*Nach Vorschrift*, as directed.*Nagel*, fingernail, toenail.*Nahe*, near, nearly.*Nase*, nose.*Nasenloch*, nostril.*Nerv*, nerve.*Neu*, new.*Neun*, nine.*Neunt*, ninth.*Neunzehn*, nineteen.*Neunzig*, ninety.*Niere*, kidney.*Nierenfett*, kidney-suet.*Notwendig*, necessary.*Oder*, or.*Oft*, often.*Ohne*, without.*Ohr*, ear.*Öl*, oil.*Paste*, paste.*Pastille*, pastille.*Pflanzenschleim*, mucilage.*Pflaster*, plaster.*Pille*, pill.*Puls*, pulse.*Pulver*, powder.*Rahm*, cream.*Recht*, right.*Reiben*, to rub in.*Reich*, copious.*Reichlich*, freely.*Rein*, clean, pure.*Rippe*, rib.*Rot*, red.*Rücken*, back.*Rückgrat*, spine.*Saft*, juice.*Salbe*, ointment, salve.*Salz*, salt.*Sanft*, soft.*Säure*, acid.*Sauerhonig*, oxymel.*Scheide*, vagina.*Schenkel*, thigh.*Schicklich*, convenient.*Schlafenszeit*, bedtime.*Schleim*, mucilage.*Schlucken*, to swallow.*Schlüsselbein*, collarbone.*Schmerz*, ache, pain.*Schneiden*, to cut.*Schnell*, promptly, quickly.*Schnitt*, cut.*Schulter*, shoulder.*Schulterblatt*, shoulderblade.*Schweineschmalz*, lard.*Schwer*, heavy.*Schwitzen*, to perspire.*Sechs*, six.*Sechsmal*, six times.*Sechst*, sixth.*Sechzehn*, sixteen.*Sechzig*, sixty.*Senden*, to send.*Sein*, his.*Seite*, side.*Selber*, *Selbe*, *Selbes*, same.*Selbst*, himself, herself, itself.*Serum*, serum.

German Glossary—continued

- Sieben*, to sift.
Sieben, seven.
Siebt, seventh.
Siebmal, seven times.
Siebzehn, seventeen.
Siebzig, seventy.
Siedend, boiling.
Sirup, syrup.
Sofort, immediately.
So oft wie, as often as.
Speise, food.
Spiritus, spirit.
Stark, strong.
Stellen, to place.
Stirn, forehead.
Stöpsel, stopper.
Stuhlzäpfchen, suppository.
Stunde, hour.
Stündlich, hourly.
Tablette, tablet.
Täfelchen, tablet.
Tag, day.
Täglich, daily.
Tassevoll, cupful.
Tausend, thousand.
Teelöffelvoll, teaspoonful.
Teil, part.
Teilen, to divide.
Tinktur, tincture.
Trank, draught.
Trinken, to drink.
Trinkglas, glass.
Tropfen, drops, drop.
Tropfen, to drop.
Über, over.
Umschütteln, to shake.
Unter, under.
Unterleib, abdomen.
Ununterbrochen, continuously.
Unze, ounce.
Vaselin, vaseline.
Verveibung, trituration.
Verdünnen, to dilute.
Viel, many, much.
Vier, four.
Viermal, four times.
Viermal des Tages, four times a day.
Viert, fourth.
Viertel, quarter.
Vierzehn, fourteen.
Vierzig, forty.
Voll, full.
Von Tag zu Tag, from day to day.
Von Zeit zu Zeit, from time to time.
Vor, before.
Vorhergehend, previous, previously.
Vorig, former.
Vorsichtig, careful, carefully, cautiously.
Wachsen, to increase.
Wachskerze, bougie.
Waschmittel, lotion.
Wade, calf.
Während, during, whilst.
Während des Tages, during the day.
Warm, hot, warm.
Wasser, water.
Wasserglasvoll, tumblerful.
Wässrig, aqueous.
Wechselweise, alternately.
Wiederholen, to repeat.
Wein, wine.
Weinglas, wineglassful.
Wenn, if.
Wenn der Husten belästigt, when the cough is troublesome.
Weite, distance, width.
Werden gemischt, to be mixed.
Woche, week.
Wahl, well.
Wofern, unless.
Zahl, number.
Zahn, tooth.
Zahnfleisch, gums.
Zehe, toe.
Zehn, ten.
Zehnmal, ten times.
Zehnt, tenth.
Zeit, time.
Zerat, cerate.
Zerreiben, to triturate.
Zu, to, on.
Zucker, sugar.
Zugpflaster, blister, plaster.
Zunehmen, to take.
Zunge, tongue.
Zusammenziehendes mittel, astringent.
Zwanzig, twenty.
Zwei, two.
Zweimal, twice.
Zweimal des Tages, twice a day.
Zweit, second.
Zwischen, between.
Zwölf, twelve.

ITALIAN GLOSSARY

- A*, on, at.
Abbondante, plenty.
Accuratamente, carefully.
Accurato, careful.
Aceto, vinegar.
Acido, acid.
Acqua, water.
Acquoso, aqueous.
Addome, abdomen.
A digiuno, fasting.
Al, to.
Alcool, alcohol.
Alcune volte, sometimes.
Alleviare, to relieve.
Alternatamente, alternately.
A meno che, unless.

Italian Glossary—continued

Anmolliente, emollient.
Ampolla di vetro, ampoule.
Anca, hip.
Antisettico, antiseptic.
Aperiente, aperient.
A peso, by weight.
Applicare, to apply.
Arnone, kidney.
Aroma, flavour.
Aromato, aromatic.
Arteria, artery.
Astringente, astringent.
Aumentare, to increase.
Avere, to have.
Bagnare, to bathe.
Bagno, bath.
Balsamo, balsam.
Bene, well.
Bevere, to drink.
Bicchiera, glass.
Bicchiera pieno, glassful.
Bicchierino, wineglassful.
Bocca, mouth.
Bollendo, boiling.
Bottiglia, bottle.
Braccio, arm.
Caldo, hot, warm.
Candela, bougie.
Capacità, capacity.
Capello, hair.
Capsula, capsule.
Casso, chest.
Cataplasma, poultice.
Catartico, cathartic.
Cautamente, cautiously.
Cena, supper.
Cento, hundred.
Cerotto, cerate.
Cervello, brains.
Cessare, to leave off.
Ciascheduno, each.
Cibo, food.
Ciglia, eyebrows.
Cinquanta, fifty.
Cinque, five.
Cinque volte, five times.
Clavicola, collarbone.
Clistere, enema.
Cloroformio, chloroform.
Colazione, breakfast.
Collirio, eyelotion.
Collo, neck.
Come, like.
Come fu detto avanti, as above.
Comodo, convenient.
Con, with.
Con acqua, in water.
Continuamente, continuously.
Continuare, to continue.
Contravveleno, antidote.
Copioso, copious.
Coprire, to cover.
Corpo, body.
Coscia, thigh.
Costantemente, constantly.

Costola, rib.
Crema, cream.
Cucchiaino, teaspoonful.
Cucchiaino da tavola, tablespoonful.
Cucchiata, spoonful.
Cuore, heart.
Da, from.
Dare, to give.
Da usarsi esterno, for external use.
Da vicino, nearly.
Decima, tenth.
Decozione, decoction.
Del, some.
Depilatorio, depilatory.
Destro, right.
Di buon'ora, early.
Diciasette, seventeen.
Diciotto, eighteen.
Dieci, ten.
Diecinove, nineteen.
Dieci volte, ten times.
Dietro, behind.
Di giorno in giorno, from day to day.
Diluire, to dilute.
Di l'un e l'altro, of each.
Di quando in quando, from time to time.
Dito, finger.
Dissolvere, to dissolve.
Diuretico, diuretic.
Dividere, to divide.
Dodici, twelve.
Dolere, pain.
Domani, to-morrow.
Dopo, after.
Dopo i pasti, after meals.
Dopo mezzogiorno, afternoon.
Doppio, double.
Dose, dose.
Dosso, back.
Dritto, right.
Due, two.
Due volte, twice.
Due volte al giorno, twice a day.
Durante, during.
Durante giorno, during the day.
Duro, hard.
Elisir, elixir.
Elletuario, electuary.
Embrocazione, embrocation.
Emetico, emetic.
Emulsione, emulsion.
Enfiagione, swelling.
Esemplare, copy.
Esterno, external.
Estratto, extract.
Essenza, essence.
Fare, to make.
Fasciata, bandage.
Fegato, liver.
Fiala, vial.
Fomentare, to foment.
Fortè, strong.
Fra, between.
Fra tanto, in the meantime.
Freddo, cold.

Italian Glossary—continued

- Frequentemente*, frequently.
Fresco, cool.
Front, forehead.
Gamba, leg.
Gargarismo, gargle.
Gargarizzare, to gargle.
Gelatina, gelatin.
Gengive, gums.
Ginocchio, knee.
Giornaliero (adj.), daily.
Giornalmente (adv.), daily.
Giorno, day.
Gocce, drops.
Gocciolare, to drop.
Gola, throat.
Gomma, gum.
Gradualmente, gradually.
Grande, big.
Granella, granule.
Grosso, big, large.
Guancia, cheek.
Il, the.
Immediatamente, immediately.
Impiastro, plaster.
In, in, into.
Infusare, to infuse.
Infusione, infusion.
Inglottire, to swallow.
Iniezione, injection.
Iniezione subcutanea, subcutaneous injection.
In luogo di, instead of.
Internamente, internally.
Intero, whole.
Intestino retto, rectum.
In tutto modo, in any case.
La, lo, the.
Labbro, lip.
Lardo, lard.
Laringe, larynx.
La tosse tormentanda, when the cough is troublesome.
Lentamente, slowly.
Liberamente, freely.
L'indomani, on the day after.
Lingua, tongue.
Linimento, liniment.
Liquido, liquid.
L'ora di andare a letto, bedtime.
Lozione, lotion.
Lubrificativo, laxative.
Male, ache.
Mandare, to send.
Mano, hand.
Mascella, jaw.
Mattina, morning.
Mattina e notte, morning and night.
Medicina, medicine.
Medesimo, same.
Membro, limb.
Mentre che, whilst.
Merenda, lunch.
Mettere, to place.
Mezzo, middle, half.
Mezzodi, midday.
Miele, honey.
Mille, thousand.
Minimo, least.
Minuto, minute.
Mischiare, to mix.
Mistura, mixture.
Misura, measure.
Misurare, to measure.
Molle, soft.
Molti, many.
Molto, much.
Mucilaggine, mucilage.
Narice, nostril.
Naso, nose.
Necessario, necessary.
Nello modo ordinando, as directed.
Nello solito modo, in the usual manner.
Nerve, nerve.
Netto, clean.
Non (adj.), no.
Nono, ninth.
Notte, night.
Novanta, ninety.
Nove, nine.
Numero, number.
Nuovo, new.
O . . . O, either . . . or.
Occasionalmente, occasionally.
Occhio, eye.
Oggi, to-day.
Ogni, every.
Ogni due ore, every other hour.
Ogni ora, every hour.
Ogni quarta hora, every four hours.
Ogni volte che, as often as.
Ogni volta che necessario, if necessary.
Olio, oil.
Omettere, to leave out.
Oncia, ounce.
Ora, hour.
Ordinazione, prescription.
Orecchio, ear.
Oso, bone.
Ottanta, eighty.
Ottavo, eighth.
Otto, eight.
Otto volte, eight times.
Parte, part.
Patella de ginocchio, kneecap.
Pasta, paste.
Pastiglia, lozenge, pastille.
Pelle, skin.
Per, by, for.
Pericraneo, scalp.
Perspire, to perspire.
Pessario, pessary.
Petto, breast, chest.
Piccolo, little, small.
Piede, foot.
Piena tazza, cupful.
Pieno, full.
Pillola, pill.
Piu, more.
Pollice, thumb.
Polmoni, lungs.

Italian Glossary—continued

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| <i>Polpa della gamba</i> , calf. | <i>Sopra</i> , over. |
| <i>Polso</i> , wrist, pulse. | <i>Sorso</i> , draught. |
| <i>Polvere</i> , powder. | <i>Sotto</i> , under. |
| <i>Ponderoso</i> , heavy. | <i>Spalla</i> , shoulder. |
| <i>Postema</i> , abscess. | <i>Spesso</i> , often. |
| <i>Pranzo</i> , dinner. | <i>Spina dorsale</i> , spine. |
| <i>Precedentemente</i> , previously. | <i>Spirito</i> , spirit. |
| <i>Prendere</i> , to take. | <i>Spiritoso</i> , spirituous. |
| <i>Presto</i> , quickly, soon. | <i>Stacciare</i> , to sift. |
| <i>Previo</i> , previously. | <i>Stesso</i> , same. |
| <i>Primiero</i> , former. | <i>Stiticità</i> , stytic. |
| <i>Primo</i> , first. | <i>Stomaco</i> , stomach. |
| <i>Principalmente</i> , chiefly. | <i>Stropicciare</i> , to rub in. |
| <i>Prontamente</i> , promptly. | <i>Subito che possibile</i> , as soon as possible. |
| <i>Prossimo</i> , next. | <i>Succo</i> , juice. |
| <i>Puro</i> , pure. | <i>Sufficiente</i> , sufficient. |
| <i>Quantità</i> , quantity. | <i>Suppositorio</i> , suppository. |
| <i>Quaranta</i> , forty. | <i>Taglia</i> , cut. |
| <i>Quarto</i> , fourth, quarter. | <i>Tagliare</i> , to cut. |
| <i>Quarto di hora</i> , a quarter of an hour. | <i>Tavoletta</i> , tablet. |
| <i>Quattordici</i> , fourteen. | <i>Tazza</i> , cup, cupful. |
| <i>Quattro</i> , four. | <i>Tempo</i> , time. |
| <i>Quattro volte</i> , four times. | <i>Tempo di mezzodi</i> , midday. |
| <i>Quattro volte al giorno</i> , four times a day. | <i>Terzo</i> , third. |
| <i>Quinto</i> , fifth. | <i>Testa</i> , head. |
| <i>Resina</i> , resin. | <i>Tiepido</i> , lukewarm. |
| <i>Respirare</i> , to breathe. | <i>Timpano</i> , eardrum. |
| <i>Respiro</i> , breath. | <i>Tintura</i> , tincture. |
| <i>Rinfrescare</i> , to cool. | <i>Tiroideo</i> , thyroid gland. |
| <i>Ripetere</i> , to repeat. | <i>Tonsilla</i> , tonsil. |
| <i>Rosso</i> , red. | <i>Tosse</i> , cough. |
| <i>Sale</i> , salt. | <i>Tre</i> , three. |
| <i>Sangue</i> , blood. | <i>Tredici</i> , thirteen. |
| <i>Sciaguatta la bottiglia</i> , shake the bottle. | <i>Trenta</i> , thirty. |
| <i>Sciaguattare</i> , to shake. | <i>Tre volte</i> , three times. |
| <i>Se</i> , if. | <i>Tre volte al giorno</i> , three times a day. |
| <i>Secondo</i> , second. | <i>Triturare</i> , to triturate. |
| <i>Sedativo</i> , sedative. | <i>Trituramento</i> , trituration. |
| <i>Sedici</i> , sixteen. | <i>Turacciolo</i> , stopper. |
| <i>Segnare</i> , to label. | <i>Ultimo</i> , last. |
| <i>Sei</i> , six. | <i>Una hora sì, l'altra no</i> , every other hour. |
| <i>Sei volte</i> , six times. | <i>Una volta</i> , once. |
| <i>Semplice</i> , simple. | <i>Undecimo</i> , eleventh. |
| <i>Senza</i> , without. | <i>Undici</i> , eleven. |
| <i>Sera</i> , evening. | <i>Un giorno di, l'altro no</i> , every other day. |
| <i>Sesto</i> , sixth. | <i>Unguento</i> , ointment, salve. |
| <i>Sessanta</i> , sixty. | <i>Uno</i> , one. |
| <i>Settanta</i> , seventy. | <i>Uretra</i> , urethra. |
| <i>Sette</i> , seven. | <i>Vaccino</i> , vaccine. |
| <i>Settimana</i> , week. | <i>Vagina</i> , vagina. |
| <i>Settimo</i> , seventh. | <i>Veleno</i> , poison. |
| <i>Sette volte</i> , seven times. | <i>Vena</i> , vein. |
| <i>Siero</i> , serum. | <i>Venti</i> , twenty. |
| <i>Sinistro</i> , left. | <i>Vesica</i> , blister. |
| <i>Sino a</i> , until. | <i>Vicino</i> , near. |
| <i>Siroppo</i> , syrup. | <i>Viscere</i> , bowels. |
| <i>Solo</i> , only, single. | <i>Zucchero</i> , sucrose. |
| <i>Soluzione</i> , solution. | |

SPANISH GLOSSARY

| | |
|---------------------------|----------------------------|
| <i>A</i> , at, on, to. | <i>Absceso</i> , abscess. |
| <i>Abdomen</i> , abdomen. | <i>Abundante</i> , plenty. |

Spanish Glossary—continued

A cada hora, hourly, every hour.

Acueo, aqueous.

Agua, water.

Agua para lavar laboca, mouthwash.

Alcohol, alcohol.

Algunas veces, sometimes.

Alimentos, food.

Aliviar, to relieve.

Alternativamente, alternately.

A mediodía, at noon.

A menos que, unless.

A menudo, often.

Ampollas de vidrio, ampoules.

Anterior, previous.

Antes, before.

Antídoto, antidote.

Antiséptico, antiseptic.

Aperitivo, aperient.

Aplicar, to apply.

Aromas, aromatic.

Astringente, astringent.

Azúcar, sucrose.

Balsamo, balsam.

Bañar, to bathe.

Baño, bath.

Baño de pie, footbath.

Beber, to drink.

Blando, soft.

Boca, mouth.

Botella, bottle.

Brazo, arm.

Bueno, well.

Bujía, bougie.

Cabeza, head.

Cada, each, every.

Cada cuarta hora, every four hours.

Cada uno, either.

Cadera, hip.

Caliente, hot, warm.

Cantidad, quantity.

Capacidad, capacity.

Capsula, capsule.

Carrilo, cheek.

Cartartico, cathartic.

Cataplasma, poultice.

Catorce, fourteen.

Cautamente, cautiously.

Cena, supper.

Cerato, cerate.

Cerca de, near.

Cercanamente, nearly.

Cercano, next.

Cesar, to leave off.

Ciento, hundred.

Cinco, five.

Cincuenta, fifty.

Cinco veces, five times.

Claro, clear.

Clavicula, collarbone.

Cloroformo, chloroform.

Colirio, eye-lotion.

Colodion, collodion.

Comida, dinner.

Como, like.

Con, with.

Con agua, with water.

Confeccion, confection.

Consecutivo, consecutive.

Constantemente, constantly.

Continuadamente, continuously.

Continuar, to continue.

Conveniente, convenient.

Copioso, copious.

Corazon, heart.

Cortaduraz, to cut.

Corte, cut.

Costilla, rib.

Crema, cream.

Cribar, to sift.

Cuando el tos agita, when the cough is

troublesome.

Cuarenta, forty.

Cuarto, fourth, quarter.

Cuatro, four.

Cuatro veces, four times.

Cuatro veces durante el dia, four times

a day.

Cubrir, to cover.

Cucharada, spoonful.

Cucharada de postre, dessertspoonful.

Cucharada de sopa, tablespoonful.

Cucharilla, teaspoonful.

Guello, neck.

Cuidadosamente, carefully.

Cuidadoso, careful.

Cutis, skin.

Dar, to give.

De, at, from.

De antemano, previously.

Debajo, under.

Decimo, tenth.

Decocion, decoction.

De dia en dia, from day to day.

Dedo, finger.

Dedo del pie, toe.

Dedo pulgar, thumb.

Del mismo modo que ya citado, as above.

De los dos, of each.

De otra manera, otherwise.

Depilatorio, depilatory.

Derecho, right.

Desayuno, breakfast.

Despues, after.

Despues las comidas, after meals.

De una vez, as soon as possible.

Dia, day.

Diario (adj.), daily.

Diente, tooth.

Dieta, diet.

Diez, ten.

Diez veces, ten times.

Diez y nueve, nineteen.

Diez y ocho, eighteen.

Diez y seis, sixteen.

Diez y siete, seventeen.

Diluir, to dilute.

Disoluir, to dissolve.

Diuretico, diuretic.

Dividir, to divide.

Doble, double.

Spanish Glossary—continued

- Doce*, twelve.
Dolor, ache, pain.
Donar, to give.
Dos, two.
Dosis, dose.
Dos veces, twice.
Dos veces durante el día, twice a day.
Durante que, whilst.
Duro, hard.
Ejemplar, copy.
El, the, himself.
Electuario, electuary.
Elixir, elixir.
Embrocacion, embrocation.
Emetico, emetic.
Emoliente, emollient.
Emplastro, plaster.
Emulsion, emulsion.
En, at, in, on.
Encarnado, red.
Encías, gums.
En el intervalo, in the meantime.
Enfriar, to cool.
Entonces, then.
Entre, between.
Enviar, to send.
Esencia, essence.
Espaldas, back.
Espina dorsal, spine.
Espíritu, spirit.
Espirituel, spirituous.
Estiptico, styptic.
Estomago, stomach.
Externo, external.
Extracto, extract.
Fomentar, to foment.
Frante, forehead.
Frasco pequeno, vial.
Frecuentemente, frequently.
Frio, cold, cool.
Frotar, to rub in.
Fuerte, strong.
Garganta, throat.
Gargarismo, gargle.
Gargarizar, to gargle.
Gelatina, gelatine.
Glicerina, glycerin.
Goma, gum.
Gotas, drops.
Gotear, to drop.
Gradualmente, gradually.
Grand, large.
Granito, granule.
Grasa, suet.
Grave, heavy.
Haber, to have.
Hacer, to make.
Hinchazon, swelling.
Hombro, shoulder.
Hora, hour.
Hoy, to-day.
Inmediatamente, immediately.
Infundir, to infuse.
Infusion, infusion.
Inhalacion, inhalation.
Interiormente, internally.
Intestinos, bowels, intestines.
Inyección, injection.
Inyección entrevioso, intravenous injection.
Inyección intramuscular, intramuscular injection.
Inyección subcutaneo, subcutaneous injection.
Izquierda, left.
Jalea, jelly.
Labio, lip.
Lactosa, lactose.
Lado, side.
La hora de acostarse, bedtime.
Langua, tongue.
Laringe, larynx.
Lavativa, enema.
Laxante, laxative.
Leche, milk.
Lentamente, slowly.
Librement, freely.
Ligero, light.
Limpio, clean.
Linimento, liniment.
Líquido, liquid.
Lleno, full.
Locion, lotion.
Mañana, morning, to-morrow.
Mañana y noche, night and morning.
Mano, hand.
Mas, more.
Medicina, medicine.
Medida, measure.
Medio, half, middle.
Mediodia, midday.
Medir, to measure.
Miel, honey.
Miembro, limb.
Mientras, during.
Mientras dura el dolor, until the pain is relieved.
Mientras el día, during the day.
Mil, thousand.
Mínuto, minute.
Mismo, same.
Mixturar, to mix.
Mucho, much.
Muchos, many.
Mucilago, mucilage.
Muneca, wrist.
Muslo, thigh.
Nariz, nose.
Necesario, necessary.
Nervio, nerve.
Ningun (adj.), no.
Noche, night.
Nono, noveno, ninth.
Noventa, ninety.
Nueve, nine.
Nuevo, new.
Numero, number.
O, or.
Ocasionalmente, occasionally.
Ochenta, eighty.

Spanish Glossary—continued

- Ocho*, eight.
Ocho veces, eight times.
Octavo, eighth.
Oído, ear.
Ojo, eye.
Omoplato, shoulderblade.
Once, eleven.
Onceno, eleventh.
Onza, ounce.
Oxímel, oxymel.
Pantorrilla, calf.
Para uso externo, for external use.
Parpado, eyelid.
Partes iguales de los dos, equal parts.
Parto, part.
Pasta, paste.
Pastilla, lozenge, pastille.
Pecho, chest.
Pelo, hair.
Pequeño, little.
Pesario, pessary.
Pericráneo, scalp.
Pie, foot.
Pierna, leg.
Pildora, pill.
Pinsel de pelo de camello, camel's hair brush.
Pocion, mixture.
Polvo, powder.
Poner, to place.
Por, by, for.
Por la mañana, in the morning.
Por la modo de, by means of.
Por peso, by weight.
Precedente, former.
Primero, first.
Principalmente, chiefly.
Prontamente, promptly.
Pronto, soon.
Pulmon, lung.
Pulso, pulse.
Puro, pure.
Quijada, jaw.
Quinto, fifth.
Receta, prescription.
Recto, rectum.
Refeccion, lunch.
Repetir, to repeat.
Resina, resin.
Respirar, to respire.
Rodillera, kneecap.
Rotular, to label.
Sacudir, to shake.
Sal, salt.
Sangre, blood.
Sedativo, sedative.
Segundo, second.
Segun se dirige, as directed.
Seis, six.
Seis veces, six times.
Semana, week.
Septimo, seventh.
Sesenta, sixty.
Sesos, brains.
Setenta, seventy.
Sexto, sixth.
Si este necesario, if necessary.
Siete, seven.
Siete veces, seven times.
Sin, without.
Sobre, over.
Solo, only, single.
Solucion, solution.
Suco, juice.
Suero, serum.
Tablilla, tablet.
Tan a menudo como, as often as.
Tarda, evening.
Tarde, afternoon.
Tapon, stopper.
Taza, cup.
Tercero, third.
Tibio, lukewarm.
Tiempo, time.
Tintura, tincture.
Todo, whole.
Todos los dias, daily.
Tomar, to take.
Tomar aumento, to increase.
Tonsila, tonsil.
Tos, cough.
Traspirar, to perspire.
Trece, thirteen.
Treinta, thirty.
Tres, three.
Tres veces, thrice.
Tres veces durante el dia, three times a day.
Trituracion, trituration.
Triturar, to triturate.
Una, uno, one.
Una vez, once.
Un cuarto de hora, a quarter of an hour.
Unguento, ointment, salve.
Un poco, some.
Vacuna, vaccine.
Vagina, vagina.
Vaselina, vaseline.
Vaso, glass.
Vejiga, bladder.
Vejigatorio, blister.
Veinte, twenty.
Vena, vein.
Venda, bandage.
Veneno, poison.
Ventana de la nariz, nostril.
Vinagre, vinegar.
Vino, wine.
Vivamente, quickly.

INDEX

As far as possible the names of substances and preparations are given in Latin. Acids are indexed under "Acid(um)." Salts are included under the Latin name of the base, and effervescent preparations under the word "Effervescent."

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